federal register

MONDAY, JULY 10, 1978
PART II

ENVIRONMENTAL PROTECTION AGENCY

REGISTRATION OF PESTICIDES IN THE UNITED STATES

Proposed Guidelines

[6560-01]

ENVIRONMENTAL PROTECTION AGENCY

[FRL 897-5]

[40 CFR Parts 162, 163, 181]

PROPOSED GUIDELINES FOR REGISTERING PESTICIDES IN THE UNITED STATES

AGENCY: Environmental Protection Agency.

ACTION: Proposed rule.

SUMMARY: This action proposes three subparts of the pesticide registration guidelines which describe the data required to support the registration of pesticide products. They would explain the overall scope, purpose, and general requirements for registration and detail the specific data requirements for product chemistry, environmental chemistry, and evaluation of hazard to wildlife and aquatic organisms.

The guidelines state the conditions under which specific data would be required to support the registration of a product, specify the standards for acceptable testing, provide references to test protocols in the scientific literature, and describe the format for reporting data. The Agency believes that adherence to these guidelines would result in a more reliable data base to support applications for registration. Furthermore, uniform testing and data submission in accordance with the guidelines will accelerate and upgrade the agency's review and evaluaton of registration applications. In addition, the Agency believes that these guidelines would aid applicants in planning the development of new and improved pesticides.

The pesticide registration guidelines are being proposed to be included in Part 163 (rather than Pt. 162) of Chapter I, Title 40 of the Code of Federal Regulations. To do so will require the following redesignations:

(a) 40 CFR, Chapter I, Subchapter E: Part 163 will have to be redesignated Part 181; and

(b) Since general Registration Procedures were published as final rules on September 9, 1975 (40 FR 41788) and codified at 40 CFR §§ 162.41-.47, this action would redesignate those rules to be Subpart C, 40 CFR §163.50-.56.

Additional subparts of the registration guidelines will be proposed in the future, including Subpart F, Hazard Evaluation: Humans and Domestic Animals; Subpart G, Product Performance; and Subpart H, Label Development.

DATES: Comments concerning these proposed guidelines should be received on or before September 8, 1978.

ADDRESS: Interested persons are invited to submit written comments on this proposed rule to the Federal Register Section, Technical Services Division (WH-569), Office of Pesticide Programs, Environmental Protection Agency, Room 401, East Tower, 401 M Street SW., Washington, D.C. 20460. Comments should bear the identifying notation OPP-30022.

Comments Availability: All written comments received under this notice will be available for public inspection in the Office of the Federal Register Section from 8:30 a.m. to 4:00 p.m., Monday through Friday.

FOR FURTHER INFORMATION CONTACT:

William H. Preston, WH-568, Criteria and Evaluation Division, Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C. 20460, 703-557-7351.

SUPPLEMENTARY INFORMATION: These reproposed guidelines are published under the authority of sections 3(c)(2), 8, and 25(a) of the Federal Insecticide, Fungicide, and Rodenticide Act, as amended ("FIFRA") (86 Stat. 973; 89 Stat. 751; 7 U.S.C. 136 et seq.) Section 3(c)(2) states that "The Administrator shall publish guidelines specifying the kinds of information which will be required to support the registration of a pesticide and shall revise such guidelines from time to time."

On July 3, 1975, the Environmental Protection Agency ("EPA", "Agency") promulgated final registration regulations, 40 CFR Part 162, Subpart A. these regulations established the basic requirements for registration of a pesticide product. Section 162.8 lists the kinds of data which the Agency would generally require to register a product.

On June 25, 1975, EPA published proposed guidelines for Registering Pesticides in the United States ("1975 proposed guidelines", 40 FR 26802). The 1975 proposed guidelines were intended to describe with more specificity the kinds of data which must be submitted to satisfy the requirements of the registration regulations. They included sections explaining the scope and intent of the guidelines, detailing the product performance, hazard evaluation, and chemistry data requirements for registration of a pesticide product, and providing guidance on proper label development.

In response to the 1975 proposal, the Agency received over 80 sets of comments from the interested public. The Agency has considered these comments in the development of this document. Because of the nature and breadth of the changes in the document, undertaken partly in response to the public comments, the Agency is today reproposing Subparts B, D, and E concerning the introduction and the data requirements on chemistry and hazard to wildlife and aquatic organisms.

The Agency intends that the guidelines provide meaningful instruction to applicants, registrants, and the general public on the specific data requirements for registration of a pesticide product. Such guidance should enable the pesticide industry to anticipate the costs and time involved in preparation and approval of an application for registration and to plan their research programs using test methods acceptable to the Agency.

Toward these ends, these proposed guidelines would specify the conditions under which each particular data requirement is applicable to a pesticide product; the standards for acceptable testing, stated with as much specificity as the current scientific disciplines can provide; and the information required in a test report. The guidelines also indicate when an applicant should consult with the Agency before initiating certain tests. In addition, the appendices to the guidelines provide useful information and references for designing test protocols and, in some cases, examples of acceptable protocols for conducting the required testing.

At present and until these guidelines are published as final rules, the data requirements for registration, the standards for acceptable testing, and the information required in a test report will be determined on a case-by-case basis. The Agency will, however, consider these proposed guidelines as a general statement of policy regarding the nature and extent of data required to support a pesticide registration.

Congress is presently considering legislation which would significantly amend FIFRA. At least one of the proposed amendments, if passed, would require revision of these guidelines. That amendment would simplify registration procedures by providing that an applicant for registration of a pesticide who proposes to purchase a registered pesticide to formulate an end-use product need not submit data on the safety of the purchase product. This amendment would significantly lessen the data requirements for many registrants of formulated products. EPA plans to publish regulations implementing the legislation soon after the bill becomes law. Among other provisions, the regulations would explain how this section of the proposed law will be implemented. Any other legislative changes affecting the guidelines will be addressed either in future amendments to this proposal or in the final version of the guidelines.

EPA's Office of Toxic Substances plans to propose testing rules under Toxic Substances Control Act ("TSCA") (Pub. L. 94-469, 15 U.S.C. 2601 et seq.) which will contain data requirements comparable to those proposed by these guidelines. In addition,

EPA or other Federal agencies and departments may issue regulations under other statutory authorities which would impose requirements similar to those in the guidelines. The Agency's policy is to reduce the burdens on the regulated public which might arise from competing requirements under the different sets of regulations and guidelines. Therefore, the Agency will establish testing rules under TSCA and guidelines under FIFRA which are consistent to the extent permitted by the different laws. The FIFRA guidelines and TSCA testing rules will also be made as consistent as possible with data or testing requirements established by other Federal regulatory bodies. If EPA publishes final pesticide guidelines and subsequently changes them to be consistent with other rules, EPA will accept data derived from testing already underway in compliance with the earlier version of the final pesticide guidelines.

I. Subpart B, Introduction to the Guidelines

The introduction sections in Subpart B contain several important new concepts and requirements which are discussed below. The requirements in Subpart B would apply to all subparts of the guidelines.

A. Revision of These Guidelines

The Agency intends these guidelines to be a dynamic document, and will provide for the periodic review of the scientific basis of all the guidelines. When necessary or desirable, the Agency will revise the guidelines in accordance with the provisions of the Administrative Procedures Act (5 U.S.C. 551 et seq.). Under that act, routine or minor changes may be made without proposal and opportunity for public comment when the Agency determines that notice and comment are impracticial, unnecessary, or contrary to the public interest. Thus, routine or minor changes in the guidelines can be made quickly. Changes to the guidelines which have significant impact would require notice and opportunity for comment. Until provisions of the guidelines have been amended, however, the Agency can implement proposed changes on a case-by-case basis, using its authority to waive data requirements, to approve nonconforming tests, and to require data from additional tests. (See pt. I C of this preamble).

B. Manufacturing-Use Product, Formulated Product, Technical Chemical, Technical Grade of the Active Ingredient

The Administrator is authorized by FIFRA to regulate those pesticide products distributed in commerce. The term "pesticide product" is defined at 40 CFR 162.3(hh) of the registration regulations to mean a pesticide offered for distribution and use; the term includes not only the pesticidal substance or mixture, but also the labeled container and supplemental labeling. Subpart B of these guidelines proposes further clarification by defining the two different kinds of pesticide products, "manufacturing-use products" and "formulated products." Section 163.40-2(d) would define a formulated product as any pesticide product "intended to be labeled with directions for end use and intended for application as registered." Section 163.40-2(c) would define a "maunfacturing-use product" as any pesticide product which was not a formulated product. Thus, a "manufacturing-use product" would refer to any pesticide product which will be reformulated; i.e., mixed with different chemical substances such as solvent to form new pesticide products, or repackaged either into smaller containers or with different labeling.

The Agency has chosen this new term, "manufacturing-use product," to replace partially another term commonly used by the industry, "technical grade product." Even though many people use the term "technical grade product" to refer to any product which does not bear an end-use label, the Agency considers that the term actually has a narrower meaning than "manufacturing-use product." "The Agency uses the term "technical grade product" to mean a product which contains only the technical grade of the pesticidally active ingredient. Most, but not all, manufacturing-use products are technical grade products; however, some manufacturing-use products are mixtures of an active ingredient with other deliberately added ingredients, and some are mixtures of two or more active ingredients. Moreover, use of the term "manufacturing-use product" would avoid the confusion which has surrounded use of the terms "technical grade product" and "technical grade of the active ingredient."

Section 163.40-2 would define this latter term. "Technical grade of the active ingredient," or "technical chemical," refers to a particular chemical substance, the active ingredient, having a certain level of purity, the technical grade. The purity of the technical chemical is not fixed by an absolute standard. It is determined, instead, by traditional industry standards, and usually refers to that identifiable, stable substance produced during the normal manufacturing process which contains the highest concentration of the active ingredient.

The use of these terms in the guidelines has a number of purposes. They are intended to assist applicants and the public in understanding the regulatory requirements which will be applied to registration applications by distinguishing between the substance tested and the product for which registration is sought. These guidelines would contain requirements to submit data to support the registration of pesticide products. The data requirements would apply to particular kinds of products; i.e., manufacturing-use products or formulated products. In addition to specifying when data are required, these guidelines would specify what data are required, and, in particular, which chemical compound must be tested. Thus, the guidelines prescribe the test substance required for each test, e.g., the technical grade of the active ingredient or the mixture comprising the formulated product.

The Agency has decided to place a greater regulatory emphasis on manufacturing-use products. The Agency thinks that manufacturing-use product registrants who produce the baisc active ingredients in pesticides for subsequent reformulation and use should be responsible for developing the basic data necessary to assess their human and environmental effects. Therefore, with the exception of the environmental chemistry sections, some data requirements apply to manufacturing-use products as well as formulated products. When a product's use pattern would practically eliminate a particular kind of risk, data assessing that risk are not required.

The major exception to the pattern of data requirements described above occurs in the environmental chemistry sections where most of the data would be required only to support the registration of formulated products. There are very real differences in how manufacturing-use products and formulated products enter the general environment. manufacturing-use products ordinarily enter the environment either through chemical plant workplace contamination or through accidents. Formulated products, on the other hand, are deliberately placed in the environment for pesticidal purposes by a wide variety of methods and in many geographical sites. Consequently, the environmental chemistry data requirements reflect the Agency's greater concern with the environmental fate and movement of formulated products.

As part of its greater emphasis on manufacturing-use products, EPA is developing a new system for handling the registration of pesticide products, called the "generic standards process." This system will assemble in a single "standard" for each pesticidally active ingredient all relevant data (concerning the effects on man and the environment) of the products containing the active ingredient. Each "standard" will also contain an explanation of EPA's regulatory position toward the

products in which the active ingredient appears. Standards will be established for manufacturing-use products first, and then they will be expanded to include formulated products as well.

The Agency requests comments on whether it would be appropriate to extend the environmental chemistry data requirements to manufacturing-use products, since much of the environmental chemistry testing must be performed with the technical grade of the active ingredient. Finally, the Agency requests comments on the clarity of the new definitions proposed in § 163.40-2, because they are used widely throughout the entire document.

C. WAIVERS OF DATA REQUIREMENTS AND DEVIATIONS FROM STANDARDS AND PROTOCOLS

The Agency fully realizes that the data requirements and standards for acceptable testing specified in these guidelines would not always be appropriate for every product. For certain products, some data requirements may be wholly inapplicable, while in other cases, additional data may be required. At times, the standards for conducting a test may have to be modified to accommodate unusual products. Therefore, 40 CFR 162.8(a)(3) of the registration regulations provides for case-by-case waivers of data requirements, and 40 CFR 162.8(d)(1) provides for imposition of additional data requirements. In addition, proposed § 163.40-3 of these guidelines would allow for deviation from the standards for acceptable testing. The Agency intends to issue waivers, require additional data, and approve deviations from standards in a reasonable manner which insures that adequate information is available and which does not over-burden applicants with irrelevant data requirements.

1. *Waivers of data requirements.* 40 CFR 162.8(a)(3) of the registration regulations contains the standard governing data waivers. It provides:

> [If] the composition, degradability, proposed patterns of use and such other properties of a specific pesticide or product * * * are *fundamentally different from the factors considered by the Agency* in establishment of the data requirements, * * * [the Agency] may waive a data requirement * * *." [Emphasis added.]

The data requirements established by these guidelines are designed to ensure that regulatory decisions are based on information which is both complete and reliable. The completeness of existing data will be considered in the decision to grant a data waiver. The factors which concern the completeness of information needed for decisionmaking are determined in part by statute. Section 3(c)(5) of FIFRA requires the Agency to make certain determinations before registering a pesticide. These determinations generally include conclusions that the product is efficacious, that its labeling and packaging conform to FIFRA, and that its use generally will not cause unreasonable adverse effects on the environment. In order to make these determinations responsibly, the Agency needs reliable information, and the guidelines data requirements have been established to assure that the relevant data are available.

Generally, all data requirements must be satisfied before the Agency will approve the registration of a pesticide product. The Agency recognizes, however, that certain pesticides may be characterized by unique physical, chemical, or biological properties or by use patterns that permit the Agency to make the statutory section 3(c)(5) determinations or other regulatory decisions without all of the data that are ordinarily required. Whether certain data are not required to support the registration of a particular product would have to be determined on a case-by-case basis.

The Agency will attempt to modify the data requirements contained in this proposal before these guidelines become final, so that waivers will be needed as infrequently as possible. Accordingly, the Agency specifically requests comment on any data requirements which should be modified or which should be waived if the requirement becomes final. The description of the proposed modification should be very specific and should be supported by a detailed rationale and appropriate data.

2. *Deviations from standards for acceptable testing; deviations from examples of acceptable protocols.* Most guidelines sections contain "standards for acceptable testing" which, as that term is defined at § 163.40-2(e), identify the factors which the Agency has determined would be critical to the reliability and completeness of the data. Appendices to the guidelines contain examples of "acceptable protocols" which, as defined at § 163.40-2(f), identify specific test methodologies which would satisfy the standards of acceptable testing.

These standards and acceptable protocols are not intended to be inflexible rules of conduct or to be the exclusive methods for meeting the specified data requirements. The Agency recognizes that the standards would not be appropriate for every pesticide product, that applicants may be aware of alternative test procedures which would be equally effective for a particular purpose, and that new procedures for deriving data will be developed in the future. Accordingly, § 163.40-3 would establish procedures for acceptance of data which deviate from the standards and suggested protocols.

Section 163.40-3(a)(1) proposes a standard governing acceptance of data which are derived from non-conforming tests: "All data required to support the registration or reregistration of a pesticide product must be derived from tests *which satisfy the purpose of the standards* for acceptable testing." [Emphasis added.] An applicant would be required to inform the Agency of every instance in which data have been derived from tests which do not conform to the standards. The Agency would then evaluate the reliability and completeness of the data and would determine whether the test procedure used satisfies the purposes of the standard. In the case of data submitted to the Agency prior to the effective date of these guidelines, the Agency may conduct this evaluation on its own initiative. (See pt. I D of this preamble.)

The acceptability of data derived from nonconforming tests would depend on a number of factors. In the first place, the Agency would have to consider the characteristics and the proposed use patterns of the pesticide product. Assuming that the standards for acceptable testing are appropriate for the pesticide product, the Agency would consider both the extent and the significance of the deviation and the particular test results. In general, the less significant the deviation from the standards, the more willing the Agency would be to accept data from tests which deviate from the standards. Furthermore, the Agency would be more willing to accept data when it appears unlikely that the particular deviation would affect the test results. An example illustrates how these two considerations can be applied.

The most commonly granted deviation from a standard will probably concern the substance tested. Many toxicology tests, such as some of those in Subpart E, must be performed on the "technical grade of each active ingredient" in the formulated product. There are sometimes several grades or brands of the same technical chemical. Even though different grades are quite similar, they usually are not chemically identical. They may differ in the kinds of contaminants and impurities present, and they may differ slightly in the concentration of the active ingredient. Thus, an applicant seeking to register a formulation manufactured with "Active A-1" may wish to rely on data resulting from tests on "Active A-2." Depending on the differences between A-1 and A-2, the Agency will either accept or reject the A-2 data as support for the A-1 product. The Agency will consider both the extent of the differences between the two and the likelihood that the differences would cause significantly different results. It must be noted that approval of a deviation for one set of test data does not necessarily mean that the Agency will accept other data de-

rived from similarly non-conforming tests. The significance of a particular deviation may vary with the pesticide under study.

Section 163.40-3(a)(2) would provide that data derived from testing which deviates from the standards for acceptable testing may be used to support regulatory actions other than approval of a registration. An example of such a regulatory action is the "rebuttable presumption against registration" process set forth at 40 CFR 162.11(a) of the registration regulations. This section provides that a rebuttable presumption against registration arises if, on the basis of tests conducted with the animal species and pursuant to the test protocols specified in the registration guidelines or by tests otherwise available, the pesticide, its metabolites, or its degradation products meet or exceed any of the criteria for risk of § 162.11(a)(3). In using data which do not conform to the standards for acceptable testing to give rise to a rebuttable presumption against registration, the Agency would have to determine that the data can serve as the basis for sound scientific and regulatory decisions. 40 CFR 162.11(a)(3) has already codified this standard of conduct by providing that:

> In making [the determination that a rebuttable presumption against registration has arisen] the Agency will take into consideration the type of effect, the statistical significance of the findings, and whether the tests were conducted in accordance with the material requirements for valid tests as recognized by experts in the field.

Nothing in these guidelines would amend that standard.

Proposed § 163.40-3 would also provide that any data submitted in support of an application for registration which satisfy the standards for acceptable testing, but deviate from a suggested protocol, would be acceptable. Applicants would be expected to use the test procedure which is most suitable for evaluation of the particular product.

D. OLD DATA

Agency files contain large amounts of data derived from testing already performed, and more data will be submitted before these guidelines become final. The Agency proposed to apply the guidelines to these old data in a commonsense manner. Since it is not in society's best interest to expend its scarce laboratory resources on redoing old tests, data will not be rejected merely because they were not developed in accordance with the guidelines test standards. Rather, the Agency will accept as valid those data which permit sound scientific judgments to be made.

E. RETENTION AND SUBMISSION OF RAW DATA, SPECIMENS, AND SAMPLES OF TEST SUBSTANCES (PROPOSED § 163.40-5)

In order to register a pesticide product, the Administrator must have available reliable scientific information pertaining to both the safety and efficacy of the pesticide concerned. Thus, section 3(c)(2) of FIFRA authorizes the Administrator to specify "the kinds of information which will be required to support the registration of a pesticide," and section 3(c)(1)(D) of FIFRA authorizes the Administrator to require an application for registration to include "a full description of the tests made and the results thereof" which are submitted in support of the application. In addition, section 8 of FIFRA authorizes the Administrator to require that certain research data be maintained and made available for inspection by the Agency.

The statutory provisions of section 3 clearly authorize the Administrator to require applicants and registrants to submit all raw laboratory data, samples of the test substance, and specimens which underlie or comprise the test or the analyses of the tests which are submitted in support of registration. Such items as daily work sheets, records, notes, and other memoranda of observations, measurements, or other laboratory activities, and specimens of animal organs or tissues, tissue blocks, or slides derived from such tests would be included. However, in the interests of both administrative efficiency and the reduction or burden upon the applicants and registrants, the Agency normally will accept, in satisfaction of the data requirements, test reports which state the procedures utilized and which summarize and interpret the resultant raw data, and normally will not require submission of the raw data, specimens, or samples of the test substance.

Nevertheless, from time to time, it may be necessary for the Agency to examine raw data, specimens, and samples of test substances to confirm the validity, accuracy, and integrity of the summary test reports actually submitted to the Agency. This need may occur on a routine basis—for example, in order to interpret or clarify a test report in the course of the Agency's initial review of an application—or may occur in connection with the Agency's toxicology data auditing program, which calls for the in-depth examination of laboratory records related to selected toxicity test reports. This audit program is designed to determine whether a test was properly performed (e.g., in accordance with its protocol), whether the raw data is internally consistent, and whether the test report submitted to the Agency fully and accurately reflects all material facts regarding both the actual test procedures and the resultant raw data.

Review of raw data, specimens, and samples is, however, complicated by the fact that many pesticide companies contract with independent testing laboratories to conduct these tests with their products. The reports ultimately submitted to the Agency by the applicant or registrant are usually prepared by the independent laboratories which often retain the raw data underlying these reports in their own archives. The matters of access to and ownership of that raw data (as between sponsor company and laboratory) may vary on an individual contract-by-contract basis. Since the Agency does not currently have authority under FIFRA to demand on-site access to raw data held by the independent laboratories or to require such laboratories to submit such raw data to EPA directly, Agency review of raw data may be seriously delayed, and efficient implementation of the auditing program or the registration process may be impeded.[1]

Accordingly, proposed § 163.40-5 would require applicants for registration and registrants to retain, in their own archives or those of a designated agent, all raw data and specimens which underlie or support any test submitted in support of an application for registration of an existing registration. The term "specimen," however, is defined to include only data submitted in satisfaction of data requirements for Subpart E (Hazard Evaluation: Wildlife and Aquatic Organisms) and Subpart F (Hazard Evaluation: Humans and Domestic Animals). In addition, an applicant or registrant would be required to retain a 20-gram sample of each test substance (other than radioactively-tagged samples). Any successor to the interest of an applicant or registrant in an application or registration also would be subject to the responsibilities of this section. The regulations: (a) would require an applicant to retain all raw data associated with an application until the application is denied; (b) would require a registrant to retain all raw data associated with a registration until the registration is cancelled; and (c) would require that specimens and samples be retained as long as their quality permits evaluation or until the application with which they are associated is denied or the registration with which

[1] In this regard, the Agency has initiated a cooperative effort with registrants concerning the audit of research records currently maintained at independent testing laboratories, and is hopeful that this effort will result in a satisfactory auditing of such records. Nevertheless, with respect to tests which will be submitted in the future in support of registration, the Agency believes that the record retention requirements proposed by this regulation are both necessary and desirable for a program of orderly and efficient review.

they are associated is cancelled. The regulations would require that all such material be stored under conditions which would minimize deterioration. They would also require the applicant or registrant (or its designated agent) to maintain an indexing and identification system which would permit him to expeditiously locate and retrieve the material. Upon request, an applicant or registrant would be required to submit or to make available to the Agency for inspection or analysis any raw data, specimen, or sample of a test substance required by this section to be retained. Under FIFRA sections 3(c)(6) and 6(b)(1), failure to make such material available to the Agency upon request is grounds for denial of the application or cancellation of the registration, as appropriate.

II. Product Chemistry

The product chemistry guidelines would require more extensive data than have been previouly required on the chemical composition of a pesticide product at the time of registration application. They would also require a commitment from registrants that the composition of the product, when manufactured and distributed, would not vary from the composition evaluated at the time of registration application. These requirements are designed to improve the reliability of the Agency's safety and efficacy decisions. This part of the preamble discusses these data requirements in more detail and examines several issues concerning these requirements.

A. Data on Manufacturing Process and Discussion of Unintentionally Formed Ingredients

Section 163.61-4 would require all applicants to begin their discussion of the composition of their products with a description of the manufacturing process, including the starting materials, the manner of combination of ingredients, and other information which pertains to the composition of the resulting product.

Industry comments already received have alleged that EPA lacks the legal authority to require such data, that EPA does not need the information to verify product composition, and that such data are trade secrets which might be revealed if they were EPA files. The Agency has not been persuaded by these arguments. The Agency has authority under FIFRA to require submission of any data, even trade secret information, which are necessary to evaluate the pesticide. Data on the manufacturing process are needed to validate the reliability of the applicant's composition statement. Only by independently evaluating the applicant's discussion of his manufacturing process can the Agency be assured that the applicant has made a thorough and diligent effort to anticipate ingredients which might reasonably appear in the pesticide product. Moreover, several industry representatives have stated (at the Scientific Advisory Panel meeting held on July 20-22, 1977) that this requirements will not force their companies to perform any additional work since this kind of description is routinely prepared during the development of a product. For these reasons, the Agency has chosen not to limit the scope of this requirment, but does solicit further comment.

In addition to the description of the manufacturing process, applicants would be required under § 163.61-5 to furnish a discussion concerning any substances which might reasonably be expected to be formed during the manufacturing process. This discussion also would address the possibility that any substance used in the manufacturing process could remain as an ingredient in the final product.

B. Composition Statement and Certified Limits

Under proposed § 163.61-3, each applicant for registration of any product would be required to list all chemical substances which he knows or might reasonably expect will be present in future marketed quantities of his product. Under proposed § 163.61-6, he would be required to declare maximum and minimum limits for each active ingredient and deliberately added inert ingredient, and maximum limits for each impurity. Then he would be required to certify that those limits would not be exceed in any quantity of the pesticide which he may sell or distribute in the future.

Section 163.61-6(b) also requires that the applicant explain the means used to establish the certified limits. The applicant may base the limits on any appropriate information, such as the results of analytical assays of the product or the calculated composition based on the anticipated reactions between known amounts of materials used in the manufacture of the product. The explanation must also discuss the accuracy and precision of the means used to establish the limits, considering, for example, the sensitivity of the analytical method or the variations in amounts of the starting materials. Using these explanations and its own independent evaluation of other information submitted by the applicant, the Agency will be able to assess the reliability of the certified limits.

Certified limits are essential for increasing the reliability of the composition statement furnished with the application. As stated in § 163.61-6(b), certified limits would tie together the registration and enforcement provisions of FIFRA. Under FIFRA § 12(a)(1)(C), it is unlawful for any person to sell or distribute:

> "any registered pesticide the compostion of which differs at the time of its distribution and sale from its composition as described in the statment required in connection with its registration under [FIFRA] section 3 * * *."

In the future, the Agency could sample products sold or distributed in commerce and analyze the samples for content of active ingredients, deliberately added inert ingredients, and impurities. If the analytical results demonstrate that any maximum or minimum certified limit has been exceeded, enforcement action may be taken. The realization of this enforcement potential should prompt applicants to furnish certified limits which provide a realistic view of the potential range of contents in products, and should encourage firms to maintain or adopt adequate quality control procedures.

The certified limits will also be used in the registration process to evaluate potential toxicity and efficacy problems. Certified limits would be especially useful for anticipating toxicity problems which were not detected by animal toxicity testing performed with samples of the product. For example, impurities for which limits have been set might not have been present in the sample upon which toxicfty tests were conducted. Similarly, the toxicity tests might have used a sample containing a lower amount of a toxic ingredient than the applicant certifies could be present in the product to be marketed. Thus, EPA would know how reliably the toxicity test had evaluated risk by comparing the substance tested with the proposed composition statement and certified limits.

C. Analytical Methods

Section 163.61-7 requires applicants to submit analytical methods for measuring the quantity of each active ingredient in a product, and sometimes the amounts of certain impurities. The Agency can use the methods to monitor the amount of chemicals in a product. If the ingredient does not conform to the certified limits, the Agency can take enforcement action against the producer of the product. In addition, the applicant must use the method to test several typical samples of the product and must report the results. The Agency will review these data both to determine whether the method is adequate for regulatory use and to determine the reliability of the certified limits.

The Agency does not require applicants to submit analytical methods for measuring the amounts of every ingredient in every product. Rather the Agency's concern is with the amounts of potentially toxic chemicals which may be introduced into the environment. Accordingly, the Agency only

requires analytical methods for those kinds of chemicals which experience has shown are most likely to pose toxicity problems.

Every pesticide product contains a pesticidally "active ingredient," which by definition produces toxic or behavior-altering effects in one or more organisms exposed to the chemical. The fact that pesticidally active chemicals can, under certain circumstances, pose hazards to man and other beneficial forms or life is, of course, well recognized. The fact that a product's active ingredients are not necessarily the only hazardous ingredients is less fully appreciated. The commercial production of pesticidally active chemicals may introduce manufacturing impurities or byproducts which are also toxic. Moreover, formulated products contain, in addition to the active ingredients and their impurities, other chemical substances called "inert ingredients," deliberately added to improve the usefulness of the product. These substances, though pesticidally inactive in the product, are not necessarily nontoxic, nor are they necessarily biologically or chemically inert. An inert ingredient may bring its own impurities, or may react with other ingredients or packaging materials to produce new impurities. Finally, some pesticides are somewhat unstable and, over time, may partially degrade to form still more substances.

Of all the different kinds of ingredients possible in the final pesticide product, EPA is most concerned with the active ingredients and the impurities associated with the manufacture of the pesticidally active ingredients. Experience has shown that the active ingredients and associated impurities are in many cases quite toxic. Though several intentionally added inert ingredients have posed toxicity problems, the number is a relatively small fraction of all inerts.

The requirements for analytical methods, therefore, are designed to reflect the Agency's greatest concerns. An applicant seeking registration of a manufacturing-use product (a product which does not bear labeling instructions for immediate use as a pesticide) would provide analytical methods for each active ingredient and each impurity which is present at a level greater than 0.01 percent of the product. However, to register most formulated products—those which are not produced by an "integrated formulation system"—an applicant ordinarily would provide an analytical method only for the active ingredients(s). (The requirements for a formulated product produced by an "integrated formulation system" are discussed below.) Finally, on a case-by-case basis, the Agency could require additional, more detailed composition data on intentionally added inert ingredients or impurities in any product.

Manufacturing-use products usually consist entirely of a technical grade of a single active ingredient. The impurities in such manufacturing-use products are the ones of primary concern to the Agency because they are produced during the course of the manufacture of the active ingredient. Consequently, analytical methods would be required for these impurities. By contrast, the Agency would not routinely require analytical methods for impurities in formulated products which are not produced by an integrated formulation system. As discussed above, the formulation impurities in these products normally are of lesser concern to the Agency. Furthermore, because the formulation of these products involves use of a registered manufacturing-use product which is subject to EPA inspection prior to incorporation into the formulated product, the Agency can easily regulate the impurities introduced with the active ingredient.

One group of formulated products, those produced by an "integrated formulation system," as defined by proposed § 163.61-1(c), are treated differently. Because the Agency has no regulatory jurisdiction over the active ingredients and their impurities before they appear in this kind of formulated product, the Agency must require, in addition to the methods for the active ingredient, methods for the impurities associated with the active ingredients. An explanation of the term "integrated formulation system" will clarify this point. An "integrated formulation system" is one in which a firm manufactures an active ingredient which it does not register but uses directly to formulate another pesticide product. EPA normally would have no analytical method on file to measure impurities associated with any active ingredient produced by this system. Even if such a chemical were also incorporated into a registered manufacturing-use product, if some of it was used to formulate an end-use product at the same facility, EPA would lack legal authority under FIFRA to obtain samples of the amount so used. Consequently, without the aforementioned special requirement, the Agency would have no way of knowing whether any such pesticide exceeded the certified impurity limits.

The most satisfactory way for the Agency to monitor the impurity contest associated with the active ingredients in products formulated by such an integrated system would be to require applicants for registration of such products to furnish analytical methods for measuring the impurities in each formulated product.

The Agency considered and rejected an alternative which would have required all formulated product applications to contain analytical chemistry methods for measuring the amounts of intentionally added inert ingredients in the product. The Agency rejected this alternative because the regulatory benefits from these data did not justify the costs of developing the methods at this time, and chose instead to allow the manufacturer to specify the method used to establish limits. As more information becomes available under the Toxic Substances Control Act, the Federal Food, Drug, and Cosmetic Act, and several other Federal sources, the Agency will reconsider these requirements. As noted above, the Agency will require additional analytical methods ony on a case-by-case basis. The Agency requests public suggestions for a more systematic way to select intentionally added ingredients for which methods should be required.

D. IDENTIFYING INFORMATION ON PRODUCTS AND INGREDIENTS

Proposed § 163.61-3 would require certain identifying information on the product and the ingredients listed in the composition statement. The applicant would be required to furnish all of the identifying names, numbers, or other information which the Agency could use to locate data relevant to a product or its ingredients during literature searches or crosschecks of its files.

E. PHYSICAL AND CHEMICAL PROPERTY DATA AND SUBMITTAL OF SAMPLES

The Agency would require data on a number of physical and chemical properties of the products and selected ingredients. Some properties such as boiling point, solubility, and melting point would be used to confirm the accuracy of the description of the manufacturing process. Other properties would be used to evaluate hazard potential, such as flammability, pH, and dielectric breakdown voltage. A third group of properties, such as odor and color, is particularly helpful to medical personnel in identifying the causative agent in accidental poisonings.

One property, the octanol/water partition coefficient, has been the subject of considerable discussion. The coefficient can be used as a crude measure of a chemical's lipid solubility and, to some extent, the capacity of the chemical to accumulate in fatty tissue. The Agency does not consider these data as a substitute for residue accumulation studies in animals [for example, the fish bioaccumulation study required by § 163.62-11(d)], because the residue studies provide information on accumulation of metabolites as well as the parent compounds, and because they provide actual, rather than theoretical, measurements of accumulation. Comments on the utility of the partition coefficient are specifically requested.

Finally, on a case-by-case basis, the Agency can also request identifying spectral data to resolve questions involving the identicality of products, and submittal of samples to verify composition statements, evaluate analytical assay techniques, or perform its own testing.

F. REQUIREMENTS FOR INERT INGREDIENTS

Nearly every pesticide product contains "inert ingredients" which are not "active" within the meaning of FIFRA section 2(m). As part of its responsibility to determine whether a pesticide causes unreasonable adverse effects on the environment, EPA intends to give greater attention in the future to the effects of inert ingredients on man and the environment. As a first step toward meeting this responsibility, the Agency is proposing more extensive data requirements in these guidelines concerning the presence of inert ingredients in pesticide products. The Agency requests public comment on whether the appropriate data are being required to permit EPA to initiate a comprehensive program for regulating inert ingredients in the future.

Proposed § 163.61-7 contains the most important new requirement for inert ingredients: an analytical method capable of measuring the impurities associated with the manufacture of an active ingredient. In addition to this information, the Agency will require applicants to make a much more thorough investigation of the composition of their products. The Agency will look very closely at the possible formation of unintentional ingredients. In addition to identifying inert ingredients, § 163.61-3 would require applicants to provide certain identifying information on the chemicals. Finally, the Agency will require, for the first time, that applicants establish and certify limits of composition for all ingredients, including inert ingredients.

Once EPA has reviewed the inert composition of a product, the Agency would determine whether additional information, such as environmental chemistry or toxicology data, is required to evaluate the product's environmental hazard. At present, the Agency plans to make these decisions on a case-by-case basis. The decisions would probably be based on the apparent toxicity of the inert ingredient, the product's use pattern, and its exposure potential.

The Agency has chosen not to adopt an alternative plan which would require testing of each inert ingredient in the same regimen of environmental chemistry and toxicological studies as are proposed for the active ingredients. Routine testing of all inert ingredients does not seem practical in light of the Nation's limited testing resources. The decision to require such data would depend upon all of the relevant information available from any source, such as, for example, that which might be developed under the Toxic Substances Control Act.

The Agency also requests public comment on its plans to impose additional requirements for environmental chemistry or toxicology data only on selected inert ingredients.

III. Environmental Chemistry

The environmental chemistry guidelines would require data on the fate of a pesticide product once it enters the environment. These data requirements generally would apply to pesticides intended for "outdoor application" as that term is defined in 40 CFR § 162.3(cc) of the registration regulations. The term includes any pesticide application that occurs outside or whose consequences would be used to define what chemical substances remain in the environment after a pesticide is used, how long and at what levels the residues persist in the environment, and where the residues are likely to be found in relation to where the product has been used.

The environmental studies, from which data would be required, are divided generally into physical and chemical degradation, metabolism, mobility, field dissipation, and accumulation. Physicochemical degradation studies identify residues which are formed through the action of water or sunlight on a product and determine the fate of those residues. Metabolism studies identify pesticide residues formed when the product is placed in water, activated sludge, or various kinds of soil. Mobility studies predict the movement of a pesticide and its residues outside the treated area, through leaching, volatility, adsorption, or dispersal by water. Field dissipation studies are conducted under actual use conditions at several sites to confirm data generated by required laboratory studies. Accumulation studies measure the propensity of a pesticide or its residues to collect in rotational crops, fish, and irrigated crops.

These environmental chemistry data would be collected and evaluated together with toxicity data to assess the potential hazards to man, fish, wildlife, or other non-target organisms resulting from use of a pesticide. These data would indicate the levels of a pesticide and its residues to which man or other non-target organisms might be exposed. Toxicity data would then be used to determine the nature of the risk created by exposure to those levels. The environmental chemistry data would also indicate whether the environmental behavior of a pesticide or its residues could lead to loss of land and water resources or could adversely affect important microbial populations.

The proposed environmental chemistry data requirements are very complex because it is necessary to evaluate the different environmental forces which can affect a pesticide. The complexity is compounded further because the specific environmental factors which act on a pesticide depend, to some extent, on how the product is used. Many commenters had difficulty understanding the data requirements of the 1975 proposed environmental chemistry guidelines. In order to express the requirements more clearly, the Agency has completely rewritten and reorganized the environmental chemistry guidelines and added a table in § 163.63-3 which summarizes the data requirements. The table presents, for each kind of use pattern, the particular tests which would be required. The specific data requirements are contained in §§ 163.62-7 through 163.62-13. Each section defines the standards under which the test should be performed; reference must be made to § 163.62-4(c) for what substance(s) must be tested.

Though much of the environmental chemistry data would be derived from tests on the technical chemical, the data are generally required only to support the registration of formulated products. As discussed in Part I-B of this preamble, the Agency is considering whether these data should also be required to support the registration of a manufacturing-use product. Such a decision would be consistent with the philosophy of the rest of these guidelines that registrants who produce a pesticidally-active ingredient for subsequent reformulation and use should be responsible for developing the basic data necessary to assess human and environmental effects. The Agency solicits public comment on this matter.

Comments on the 1975 proposed guidelines on environmental chemistry, and internal and external review of earlier drafts of this proposal, have identified a number of major issues which are discussed in the following paragraphs. The Agency solicits further public comments and suggestions concerning each of these issues. The Agency requests that each comment which suggests deleting or modifying a requirement be accompanied by a rationale and an example of an alternative methodology, if appropriate.

A. SOIL PHOTODEGRADATION

Section 163.62-7(c) would require data on the photodegradation of a pesticide on soil surfaces. The Agency would use the data to identify residues formed by sunlight and to evaluate the significance of photodegradation as a mechanism for pesticide decomposition in soil. Industry comments already received have alleged that the

study is difficult to perform, that the results are hard to interpret, and that the same information may be derived from a 14 carbon plant metabolism study conducted under field or growth chamber conditions. They have also suggested that photodegradation on soil surfaces only accounts for a small amount of the degradation of a product in soil, and consequently data are not very important. The Agency's experience is that soil photodegradation studies are less expensive and easier to perform and to interpret than photolysis studies in other systems. Moreover, the Agency is familiar with several pesticides which are degraded significantly by the action of sunlight on soil surfaces. The Agency solicits public comments on the proposed soil photodegradation study. The Agency also requests comments on the adequacy of the suggested alternative 14 carbon plant metabolism study to provide equivalent information, especially for crop and forest uses.

B. EFFECTS OF MICROBES ON PESTICIDES

Various people have recommended deletion of § 163.62-8(f)(2), which would require data on the effects of microbes on pesticides. Based on its experience, the Agency believes that these proposed studies are needed to identify (if possible) the kinds of microorganisms which are responsible for metabolism of a pesticide and the relative contribution of microbial metabolism to degradation of a pesticide. The Agency is aware of several pesticides which are degraded primarily by microorganisms with a limited environmental range. If these microbes were unavailable to degrade the pesticide (for example, due to the presence of a toxicant), the persistence of the product would change markedly. As the persistence increases, so could the environmental hazard.

Industry comments already received suggest that the soil metabolism studies proposed in § 163.62-8 (b) and (c) could be modified to include sterile and non-sterile conditions. The resulting data would allow the Agency to evaluate, to a limited extent, the relative importance of microbial degradation of a pesticide. Such data, however, would be applicable only to the terrestrial uses of a pesticide and would not indicate how a pesticide might behave in an aquatic system. Moreover, the data would not indicate which genera of microorganisms were active in degrading the product. The Agency specifically solicits comment on the proposed requirement and the suggested alternative.

C. EFFECTS OF PESTICIDES ON MICROBES

Section 163.62-8(f)(3) would require data on effects of pesticides on microbes. The Agency would use these data to detect potential adverse effects on microorganisms which could have significant consequences for man, fish and wildlife, or the environment. Data from terrestrial microbial studies would indicate whether a pesticide could interfere with the nitrifying functions of soil microbes or could materially reduce the numbers of nitrogen-fixing bacteria with a concurrent increase in numbers of fungi. These two effects could lead to a reduction in overall soil fertility. Properly chosen studies of pesticidal impact on microbes could also indicate potential adverse effects on the overall health of an ecosystem. For example, in an aquatic system the studies should measure the effects of a pesticide on bacteria and algae, which support directly or indirectly all higher organisms in the ecosystem. Similar studies could be performed with appropriate terrestrial microorganisms. Finally, data from these studies could be very valuable in supplementing the data from studies of the effects of microbes on pesticides. If data indicated that degradation of a particular pesticide depends on the activity of a certain microbial population, these studies could be designed to determine whether other pesticides would inhibit those microbial populations. Based on such data, the Agency could prepare label warnings against use of a microbial toxicant in areas where it would interfere with degradation of other pesticides. The Agency solicits further public comment on the utility of requiring data from this study for registration purposes.

D. ACTIVATED SLUDGE METABOLISM STUDY

Section 163.62-8(g) would require an activated sludge metabolism study for all products which are indirectly discharged into the aquatic environment or directly discharged into wastewater treatment systems. The requirement for sludge metabolism data did not appear in the 1975 proposed guidelines. The purpose of the study is to determine whether a pesticide is capable of disrupting the operations of a sewage treatment facility by preventing or severely retarding the decomposition of sewage. The Agency knows of several pesticides such as kepone, and other chemicals such as polychlorinated biphenyls (PCB's), which have inhibited sewage decomposition and thus allowed nondecomposed sewage to contaminate the aquatic environment. The Agency expects that if data from activated sludge studies were available, the number of incidents could be reduced in the future. The Agency requests public comments on this proposed requirement, and particularly solicits recommendations for improved methodology.

E. LEACHING STUDIES

Comments already received have questioned the need for data on the leaching properties of pesticides. (See § 163.62-9(b).) The comments suggest that the leaching of a chemical may be adequately determined by data on the adsorption/desorption coefficients of the chemical and its metabolites. Other comments have suggested, however, that adsorption/desorption is not always the most significant mechanism influencing leaching, and thus data from leaching studies are needed. The Agency requests public comment on these proposed requirements.

F. ROTATIONAL CROPS

Section 163.62-11(b) would impose requirements for data on pesticide residues in rotational or subsequently planted crops that would be more extensive than those in the 1975 proposed guidelines. The guidelines would require the applicant to study residue uptake in representative root, grain, and leafy vegetable crops. Distribution of crops with such residues would be illegal under the Federal Food, Drug and Cosmetic Act unless a tolerance had been established and the residue did not exceed the tolerance. However, no routine mechanism exists for establishing tolerances for residues in rotational crops under the current tolerance-setting procedures. Instead, when no tolerance has been approved and the Agency expects residues could occur in a rotational crop, it has required label restrictions against planting that crop.

The Agency is currently reevaluating its tolerance-setting procedures and requests public comment on both the proposed data requirement and the various regulatory mechanisms to control pesticide residues in rotational crops.

G. FUTURE REQUIREMENTS—DATA FOR ASSESSING SAFE REENTRY INTERVALS AND DATA TO SUPPORT LABEL DIRECTIONS ON DISPOSAL AND STORAGE

Section 163.62-12 is reserved in the present proposal. When proposed, it will contain requirements for environmental chemistry data which would be used with other data to establish safe reentry intervals. The Agency plans to propose all sections which would establish data requirements relating to reentry intervals at the same time. These sections will include proposed requirements for toxicity data and exposure evaluation, as well as environmental chemistry data.

Proposed § 163.63-13 will be revised at some future time to establish specific data requirements concerning the disposal and storage of pesticides. The Agency would use these data to develop label directions for proper storage and disposal by a user of excess pesticides and pesticide containers. Excess pesticides would mean quantities of a product, tank mixture, or rinse solution which could not be sold legally

under FIFRA and which the user intends to discard. Data on disposal could include incineration parameters, descriptions of chemical degradation methods, and the effects of injecting excess pesticides into the soil. Storage data could include measurements of vapor loss of a pesticide from its container.

The Agency requests public comments on these possible data requirements, and specifically solicits suggestions for appropriate methodology for these requirements and for any other appropriate related requirements.

H. DISCONTINUED REQUIREMENTS—BOUND RESIDUE DATA

One section of the 1975 proposed guidelines, § 162.79(a)(2)(vi), contained requirements for bound pesticide residues in soil. (A bound residue is any amount of unextractable residue, greater than 10% of the parent compound, remaining one year after a single treatment.) The 1975 proposal required extensive information on the nature and significance of binding mechanisms, potential for buildup, binding capacity of soils, and action in aerobic sites. In response to public comment, this requirement has been limited to data on the quantities of unextractable residues which are determined as part of the material balance, and the requirements for the other data have been eliminated.

I. BENCHMARK CHEMICAL CONCEPT

The proposed guidelines represent one of several possible approaches to evaluating the environmental behavior of a pesticide product. These guidelines would require an applicant to submit data from a series of laboratory and field tests on the pesticide he seeks to register. Most of these studies would attempt to quantify the product's behavior in a typical environmental situation. For example, the applicant would normally be required to submit data on persistence of residues in a soil typical of a location where the product would commonly be used.

An alternative conceptual approach for evaluating the environmental behavior of a pesticide is called the "benchmark chemical concept". Under this approach, an applicant would make laboratory measurements of certain "key environmental properties" (e.g., water solubility, vapor pressure, decomposition in soil) of a pesticide. These data would then be compared to similar measurements on established "benchmark" chemicals whose behavior under actual use conditions is well known. By matching the new pesticide with a benchmark chemical which it closely resembled, environmental behavior of the pesticide may be predicted qualitatively. (See Goring, C.A.I., J.W. Hamaker, and D.A. Laskowski, 1973. A proposed approach to the PR 70-15 guidelines. In "Chemicals, Human Health, and the Environment," Vol. I (E. H. Blair and C.E. Otis, eds.), the Dow Chemical Co., pp. 7-14.)

Although the benchmark chemical concept may be used as a screening tool or to predict qualitatively the behavior of a pesticide in the environment, the Agency has a number of reservations about relying exclusively on this approach. First, the Agency does not consider the "key environmental properties," by themselves, adequate for predicting the behavior of a pesticide under actual use conditions. Second, the Agency recognizes that the actual field behavior data of the benchmark chemicals under all possible use conditions simply is not available to the Agency at present. To use the benchmark concept effectively, the Agency would need such data on every combination of a pesticide chemical, use pattern, and environmental system.

Third, the benchmark concept is useful only to the extent that the products being compared to "benchmark" chemicals resemble any of those chemicals. When a unique chemical or use pattern is proposed, the benchmark approach would not be helpful. Finally, the benchmark approach would ignore aspects of the tests in the proposed guidelines which evaluate environmental toxicity of pesticide residues. For example, these proposed guidelines would require data on the potential of a pesticide to affect land and water resources or to reduce microorganism populations. The benchmark concept would not generate comparable data.

The Agency solicits public comment on these alternative concepts and on which aspects of the benchmark chemical concept could be merged into the proposed guidelines.

IV. HAZARD EVALUATION: WILDLIFE AND AQUATIC ORGANISMS

The guidelines for fish and wildlife hazard evaluation would require data on the hazards to birds, aquatic organisms, and wild mammals. The data required by these sections would represent only a portion of the data considered by the Agency in evaluating risks to fish and wildlife. For example, the Agency also considers the probable routes and levels of exposure indicated by the product chemistry and environmental chemistry data. And, in addition to the two tests on mammalian toxicity included in this subpart, the Agency routinely examines the mammalian toxicity data submitted for evaluation of hazards to humans and domestic animals. (See Subpart F guidelines.)

The data requirements would be arranged in a tier fashion, indicated by the figures contained in § 163.70-(a). Simple short-term laboratory studies would be required at the first level for all manufacturing-use products and for formulated products intended for outdoor application. A second level of longer term tests would be required for certain pesticides intended for outdoor application, depending on the probable toxicity demonstrated by the first level tests, and on the probable environmental exposure demonstrated by environmental chemistry data. If, based on the data generated by the second level tests, the Agency suspects that unreasonable adverse effects on fish or wildlife may occur, the third level tests would be required. These tests would evaluate the risk of a pesticide use under simulated or actual field conditions. The Agency would not routinely require data from third level tests because most questions concerning risks could be answered by data from the first and second level tests.

The fish and wildlife guidelines, like the other proposed sections, contain standards for acceptable testing and appendix references. The Agency plans to apply the standards for acceptable testing to test data in the same fashion in the fish and wildlife area as in other areas. (See discussion in part I D of this preamble.) The Appendices following most of the fish and wildlife sections contain citations to published literature which would provide examples of acceptable test protocols or background information useful in developing acceptable protocols.

The fish and wildlife guidelines also contain the text of several acceptable protocols. These protocols are contained in the guidelines because they are not available in any other published form and because they describe examples of tests which would be routinely required for a significant number of products. The Agency has chosen to publish these protocols in the guidelines rather than in a separate monograph, because EPA believes it is the most efficient way of providing this useful information to all potential users. The applicant is responsible for using a test procedure which is suitable for evaluation of a particular product, and he may use any other protocol which satisfies the standards for acceptable testing. The Agency would treat these protocols in the same manner as protocols which are contained in the appendix references. Failure to follow a suggested protocol would not invalidate a test if another appropriate and acceptable methodology had been used. (See part I C of this preamble.)

Comments on the 1975 proposed guidelines, and internal and external reviews of earlier drafts of this proposal, have identified a number of issues which are discussed in the following

paragraphs. The Agency solicits further public comments and suggestions concerning each of these issues.

A. SELECTION OF AVIAN SPECIES

There has been considerable discussion of the Agency's choice of species for avian tests. Generally, the standards for acceptable testing would require use of captive reared specimens of one or more of the following species: wild mallard duck, native bobwhite quail, and ring-necked pheasant. Commenters have suggested that the domestic duck, Japanese quail, and domestic chicken could be substituted for each of the respective required species.

The Agency is not persuaded that these species would be preferable. First, the domestic duck, chicken, and Japanese quail are generally less sensitive to a broad range of pesticides than the species for which they would substitute. Thus, use of these species would build a bias toward apparent safety. Second, the wild species which would be required by the proposed guidelines are more representative of the species at risk in the United States.

The ring-necked pheasant is the most widely-bagged game bird. The most plentiful southern game bird is the bobwhite quail, and the most common waterfowl is the mallard. By contrast, neither the chicken, domestic duck, nor Japanese quail is established as a wild species in the United States. Finally, the purchase and rearing costs of the captive wild species are as low as or lower than the comparable costs of the suggested substitute birds. The Agency welcomes additional comments on this issue.

B. NUMBER OF SPECIES IN THE AVIAN LD50 STUDY

Section 163.71-1(b)(2) would require that tests to determine the acute oral LD50 for birds be conducted using one avian species. Comments already received have suggested, on the one hand, that the test be eliminated, and, on the other hand, that two species be tested. The Agency thinks that testing one species is both necessary and adequate to generate the needed data on acute toxicity to birds.

The avian LD50 study is an important test which is useful for a number of purposes, particularly as a measure of the dose which is likely to be lethal. The LD50 for one species is indicative of the probable range of the LD50 for many other birds. The Agency also compares it with the LC50 five-day dietary data, to evaluate the possibility of cumulative toxic effects. Because the LD50/LC50 ratio is relatively comparable across avian species, the Agency thinks that one species is usually adequate to determine the approximate value of the ratio.

C. AGE OF BIRDS

Comments have questioned the age range proposed in the avian testing standards and suggested protocols. (See §§ 163.71-1(b)(3), 163.71-2(b)(4), 163.71-4(b)(4), and the accompanying suggested protocols.) Commenters have suggested that the Agency could expand the age ranges without sacrificing the sensitivity in the tests. They state, furthermore, that testing younger or older birds would be easier, would yield comparable or substantially better test results, and, in some cases, would be less expensive. The Agency is proposing the stated age ranges to maximize the sensitivity of the test, and to facilitate the comparison of data from tests on different compounds.

D. SMALL PEN FIELD TEST PROTOCOL

The agency is aware of a number of deficiencies in the protocol proposed for small pen field studies with birds. (See § 163.71-5) The protocol fails to address the need to supplement the birds' diet with untreated feed and water to evaluate repellency. Also the recommended 20-square foot pens would not permit the birds to feed naturally or to exhibit normal social and sexual behavior. Finally, the protocol would not provide the birds with adequate protection from predators. EPA is presently assembling a group of experts to develop a more appropriate protocol which should solve these problems. Once the new protocol is completed, the Agency plans to test the methodology. EPA hopes to have a new validated protocol available by the fall of 1978. The Agency solicits suggestions for modifying the existing protocol, and requests that suggestions be supported by rationales and data, if available.

E. "WHEN REQUIRED" PARAGRAPHS FOR ADVANCED STUDIES

The Agency intends that the need for data submittal required by §§ 163.71-4, -5, and §§ 163.72-4, -5, -6 be relatively infrequent. Comments have indicated, however, that the proposed criteria for when such data are required seem to be too encompassing, and that registrants would feel obligated under these criteria to conduct such studies for most chemicals. The Agency solicits suggestions for appropriate criteria that reflect more exacting needs for such tests.

V. REPORTING IMPACT AND ECONOMIC IMPACT ANALYSES

The Agency has prepared a Reporting Impact Analysis on Subparts B, D, E, and F of the proposed guidelines, conducted in accordance with the Federal Reports Act of 1942, the Federal Records Act of 1950, and the Office of Management and Budget Circular A-10. The analysis describes the record keeping and reporting requirements of the guidelines. It discusses the frequency of reporting, collection methods, alternative methodologies for acquiring equivalent information, changes in record retention requirements, and the incremental costs of change.

The Agency has also prepared an Economic Impact Analysis, covering Subparts B, D, E, and F of the proposed guidelines, and conducted in accordance with Executive Orders 11821 and 11949, OMB Circular A-107, and the Administrator's guidelines for conducting economic impact analyses on major proposals. The analysis discusses the incremental burden on registration and reregistration arising from the proposed subparts. It also includes an estimate of the cost of reregistering all presently registered products under the existing (as of Sept. 1, 1977) data requirements and under the proposed guidelines. Finally, it discusses some economic impacts that would be expected to result from promulgation of these proposed guidelines.

Copies of the Reporting Impact Analysis and the Economic Impact Analysis will be made available to interested persons, who can write the Federal Register Section at the address given at the beginning of this document. The Agency welcomes comment on these analyses.

NOTE.—The Environmental Protection Agency has determined that this document does not contain a major proposal requiring preparation of an inflationary impact statement under Executive Orders 11821 and 11949, and OMB Circular A107.

Dated: June 27, 1978.

BARBARA BLUM,
Acting Administrator.

1. In 40 CFR, Chapter 1, Subchapter E, Part 163 is redesignated as Part 181.

2. In 40 CFR, Chapter 1, Subchapter E, §§ 162.41 through 162.47 are redesignated as Part 163, Subpart C, §§ 163.50 through 163.56.

3. It is proposed that Part 163, Chapter I, Title 40 of the Code of Federal Regulations be amended by adding new Subparts B, D, and E to read as follows:

Subpart A

Sec.
163.1 through 163.39 [Reserved].

Subpart B—Introduction to the Guidelines

163.40-1 Scope and intent.
163.40-2 Definitions.
163.40-3 Deviations from test standards and acceptable protocols.
163.40-4 Biological control agents.
163.40-5 Retention and submission of raw data, specimens, and samples of test substances.
163.40-6 Disclaimer for named products and devices.

Subpart C—Registration Procedures

163.50 General information.
163.51 Registration forms.
163.52 Preparation of application packages.
163.53 Labeling.
163.54 Supporting data.
163.55 Registration review.
163.56 Disposition of applications.

Subpart D—Chemistry Requirements

163.60-1 Overview.

PRODUCT CHEMISTRY

163.61-1 Definitions.
163.61-2 Scope of requirements.
163.61-3 Product identity and disclosure of ingredients.
163.61-4 Description of manufacturing process.
163.61-5 Discussion on formation of unintentional ingredients.
163.61-6 Declaration and certification of ingredient limits.
163.61-7 Product analytical methods and data.
163.61-8 Physical and chemical properties.
163.61-9 Submittal of samples.
Appendix to Product Chemistry

ENVIRONMENTAL CHEMISTRY

163.62-1 Purpose: Hazard assessment.
163.62-2 Definitions.
163.62-3 Scope of requirements.
163.62-4 Basic standards for testing.
163.62-5 Reporting of data.
163.62-6 Use patterns associated with data requirements.
163.62-7 Data requirements: Physicochemical degradation.
163.62-8 Data requirements: Metabolism.
163.62-9 Data requirements: Mobility.
163.62-10 Data requirements: Field dissipation.
163.62-11 Data requirements: Accumulation.
163.62-12 Data requirements: Reentry [Reserved].
163.62-13 Data requirements: Disposal and storage.
Appendix to Environmental Chemistry

SPECIAL CHEMISTRY REQUIREMENTS

163.63-1 Tolerance clearance.
163.64-1 Pesticides in tobacco.
Appendix to Special Chemistry Requirements

Subpart E—Hazard Evaluation: Wildlife and Aquatic Organisms

163.70-1 General requirements.
163.70-2 Definitions [Reserved].

AVIAN AND MAMMALIAN TESTING

163.71-1 Avian single dose oral LD50.
163.71-2 Avian dietary LC50.
163.71-3 Mammalian acute toxicity.
163.71-4 Avian reproduction.
163.71-5 Simulated and actual field testing for mammals and birds.

AQUATIC ORGANISM TESTING

163.72-1 Fish acute LC50.
163.72-2 Acute toxicity to aquatic invertebrates.
163.72-3 Acute toxicity to estuarine and marine organisms.
163.72-4 Embryo larvae and life-cycle studies of fish and aquatic invertebrates.
163.72-5 Aquatic organism toxicity and residue studies.
163.72-6 Simulated or actual field testing for aquatic organisms.

AUTHORITY: Secs. 3, 8, and 25(a)(1) of the Federal Insecticide, Fungicide, and Rodenticide Act, as amended (Pub. L. 92-516, 86 Stat. 973; Pub. L. 94-140, 89 Stat. 755; 7 U.S.C. 136 et seq.).

Subpart A

§ 163.1-163.39 [Reserved]

Subpart B—Introduction

§ 163.40-1 Scope and intent.

(a) The legislative authority for pesticide registration is the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) as amended (86 Stat. 973; 89 Stat. 751; 7 U.S.C. 136 et seq.). The Administrator of the Environmental Protection Agency is authorized by section 3(c)(5) of FIFRA to register a pesticide if he determines that, when considered with any restrictions imposed under FIFRA section 3(d):

(1) Its composition is such as to warrant proposed claims for it; (2) Its labeling and other material to be submitted comply with the requirements of the act; (3) It will perform its intended function without unreasonable adverse effects on the environment; and (4) When used in accordance with widespread and commonly recognized practice it will not generally cause unreasonable adverse effects on the environment.

If the Administrator determines that all of these requirements are satisfied, the registration application will be approved. (See § 162.7(d) of this chapter). To permit this determination, the applicant for registration of a pesticide must provide data defining its composition, establishing its efficacy, and evaluating the hazards posed by its intended use(s).

(b) The regulations on the registration, reregistration, and classification of pesticides, Part 162, Subpart A of this chapter, establish some procedures and the broad data requirements for reregistration. The guidelines should be read in conjunction with these regulations.

(c) Section 3(c)(2) of FIFRA provides that "The Administrator shall publish guidelines specifying the kinds of information which will be required to support the registration of a pesticide." Subparts B-H of Part 163 accomplish this purpose.

(d) The purposes of the guidelines are: (1) To provide pertinent information and instruction to both applicants for registration and interested members of the general public relative to six major subject areas:

(i) Current registration procedures; (ii) Data and labeling requirements for registering pesticides; (iii) The standards for acceptable testing; (iv) Performance standards for product efficacy; (v) Acceptable test methods for the development of required data; and (vi) The information required in test reports.

(2) To enable registrants to improve the quality, completeness, and consistency of applications for registration, thus permitting registration officials to review submitted applications expeditiously; and

(3) To assure that both data development and review are based on a uniform set of standards.

(e) Subpart C, Registration Procedures, pertains to the procedures that applicants and registrants must undertake to obtain initial registration of a pesticide, to amend a registration, to renew a registration, and to obtain reregistration and classification of pesticide products to bring them into conformity with the amended FIFRA.

(f) Subparts D, E, F, and G pertain to the data on chemistry, hazard evaluation, and product performance, respectively, which are required to support an application for registration. These data encompass three major areas of Agency concern: efficacy, human safety, and environmental safety. These supporting data are required to enable the Agency to make judgments as to acceptability of products for registration. Such data are related to claims, directions for use, limitations, precautions, and other statements made on the pesticide product labeling.

(g) Subpart H, Label Development, pertains to the requirements for labels of pesticide products and for all other labeling that accompanies the product at any time. Specific instructions and advisory information regarding labeling for the various types of pesticide products is included.

(h) The validity of information submitted to meet data requirements depends to a great extent on the test procedures employed and the expertise of the individuals performing and evaluting the tests. The guidelines set forth standards for acceptable testing, stated with as much specificity as the current scientific disciplines can provide.

(i) These guidelines include appendices providing examples of or references to test methodology which, if followed properly, can produce data satisfying the standards for acceptable testing.

(j) Neither the standards for acceptable testing nor the examples of acceptable protocols are to be construed as inflexible and exclusive for meeting the specified data requirements. The Agency recognizes that applicants may be aware of other test procedures which are equally effective for particular purposes, and that new procedures will be developed in the future. It also recognizes that the indicated test methods may not be suitable for the evaluation of a particular product.

Thus, data from tests which deviate from the test standards and protocols may be acceptable. (See § 163.40-3.)

(k) Neither the guidelines nor the appendices are static documents. Section 3(c)(2) of FIFRA states that the Administrator "shall revise such guidelines from time to time." Therefore, the guidelines and appendices will be expanded and revised periodically to reflect new scientific knowledge, new trends in pesticide development, and new Agency policies. All applicants for registration and new applicants, particularly, are encouraged to consult the Agency to resolve any questions relating to the guidelines or the data requirements for registration. Any applicant who believes that a data requirement of subpart B through H is inapplicable to a specific pesticide or product may petition the Administrator for waiver of a data requirement in accordance with § 162.8(a)(3) of this chapter.

(l) An applicant may satisfy the requirements contained in these guidelines either by submitting the required information or by referencing data previously submitted to support the registration of another product. (See § 162.9 of this chapter.)

(m) Applicants are reminded that statutes other than FIFRA may affect the regulation of pesticides. Specifically, producers of pesticides subject to registration under FIFRA may also be subject to provisions of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. 321 et seq.; the Federal Water Pollution Control Act, 33 U.S.C. §§ 1251 et seq.; the Federal Hazardous Substances Act, 15 U.S.C. §§ 1261 et seq.; the Occupational Safety and Health Act, 29 U.S.C. §§ 651 et seq.; the Resource Conservation and Recovery Act of 1976, 42 U.S.C. §§ 6901 et seq.; and the Toxic Substances Control Act, 15 U.S.C. §§ 2601 et seq. Specific references to appropriate statutes and their relevance to registration matters are made in other parts of these guidelines.

§ 163.40-2 Definitions.

Terms used in subparts B-H shall have the meaning set forth in FIFRA and § 162.3 of this chapter. In addition:

(a) "Technical grade of active ingredient" means a substance (1) Which contains an active ingredient; (2) Which is produced on a commercial scale for use in pesticide manufacture, or on a pilot-plant scale; and (3) to which no inert ingredient has been deliberately added for any purpose other than synthesis, extraction, or purification of the active ingredient. This term is synonymous with the term "technical chemical."

(b) "Pesticide formulation" means the substance or mixture of substances comprised of all active ingredients and inert (if any) ingredients of a pesticide product.

(c) "Manufacturing-use product" means any pesticide product other than a formulated product, including, for example, a product intended for use as a pesticide after (re)formulation or repackaging, and a pesticide product intended to impregnate articles which are exempt from the registration requirement pursuant to § 162.4(c) of this chapter.

(d) "Formulated product" means a pesticide product intended to be labeled with directions for end use and intended for pesticidal application as registered.

(e) "standards for acceptable testing" means the factors which are critical to the validity and reliability of the testing. This term is synonymous with the term "test standards."

(f) "Acceptable protocol" means a specific test methodology which satisfies the standards for acceptable testing.

§ 163.40-3 Deviation from test standards and acceptable protocols.

(a) *Deviation from standards.* In some circumstances the Agency will take regulatory action with respect to a pesticide product based on data which are derived from tests which do not conform to the standards for acceptable testing.

(1) *Approval of registration.* All data required to support the registration or reregistration of a pesticide product must be derived from tests which satisfy the purposes of the standards for acceptable testing. After the effective date of these guidelines, consultation with the Agency before using test procedures which would not conform to the standards is strongly recommended.

(i) *Notice of deviation.* An applicant who submits data after the effective date of these guidelines in support of the registration or reregistration of a pesticide product must inform the Agency of every instance in which the data have been derived from tests which do not conform to the standards for acceptable testing.

(ii) *Procedure for evaluation nonconforming tests.* When an applicant for registration or reregistration seeks to rely on data derived from a test which does not conform to the test standards, he may submit written evidence that the test procedures used satisfies the purpose of the standard in question. After considering the evidence submitted by the applicant, and such other information as may be available, the Administrator will make a written finding with respect to whether the test procedure used satisfied the purposes of the standard. The Administrator may, on his own initiative, make such a finding in the case of data previously submitted to the Agency.

(2) *Regulatory actions other than approval of registration.* Data derived from testing which deviates from the standards for acceptable testing may be used in support of regulatory actions other than approval of a registration; e.g., see § 162.11(a)(3) of this chapter.

(b) *Deviation from suggested acceptable protocols.* Any protocol which satisfies the standards for acceptable testing may be used. Applicants are required to use the use the test procedure which is most suitable for evaluation of the particular product.

§ 163.40-4 Biological control agents.

The data requirements for those living organisms or viruses which are pesticides will be determined on a case-by-case basis after consultation with Agency.

§ 163.40-5 Retention and submission of raw data, specimens, and samples of test substances.

(a) *Definitions.* For the purposes of this section: (1) The term "raw data" means all laboratory or field work sheets, records, notes, or memoranda that are the result of observations, measurements, or other activities performed during the testing or evaluation of a pesticide, and all evaluations and analyses thereof, including correspondence pertaining to planning, conduct, or interpretation of the study.

(2) The term "specimen" means any preserved organ or tissue, tissue block, or slide which was examined or analyzed during the testing or evaluation of a pesticide, as required by subparts E and F of this part.

(b) *Retention.* (1) Each person who generates and submits data or summaries of data to the Agency in support of a registration or an application for registration, and any successor to the interest of such a person in the registration or application for registration, shall retain:

(i) All raw data and all specimens which pertain to such data; and (ii) a 1-gram sample of any analytically pure test substance used in test(s) and a 20-gram sample of any other test substance used in test(s) (e.g., technical grade of the active ingredient, formulated product, or other substance whose effects were studied). Readioactively-tagged samples of the test substance do not need to be retained for the purpose of the requirements of this section.

(2) All raw data shall be retained until each application with which they are associated is denied or until each registration with which they are associated is cancelled.

(3) Specimens and samples of the test substance shall be retained for as long as the quality of the material permits evaluation, or until the application with which they are associated is

denied, or until the registration with which they are associated is canceled.

(c) *Submission.* (1) Upon request by the Agency, the applicant or registrant shall submit or make available to the Agency for inspection or analysis any raw data, specimen, or sample of a test substance required to be retained by this section.

(2) No applicant or registrant shall submit any raw, data, specimen, or sample of a test substance to the Agency unless:

(i) Specifically requested to do so, in writing, by the Agency; or (ii) Specifically required to do so, by Agency regulations.

(d) *Failure or refusal to submit; cancellation or denial of registration.* (1) Failure or refusal by an applicant or registrant to submit or to make available for inspection or analysis, upon request by the Agency, any raw data, specimen, or sample of test substance requires to be retained by this section constitutes grounds for the denial of the application with which they are associated, under section 3(c)(6) of the act, or for the cancellation of the registration with which they are associated, under section 6(b)(1) of the act.

(2) If the Administrator issues a notice of intent to deny an application or cancel a registration in accordance with paragraph (d)(1) of this section, the applicant, registrant, or other affected party, as provided by law, may request a hearing in accordance with section 3(c)(6) or section 6(b)(1) of the act and part 164 of this chapter.

(i) Any such hearing shall be limited to the following issues for which the petitioner shall have the affirmative burden of proof:

(A) Whether the applicant or registrant submitted or made such material available to the Agency upon its request;

(B) Whether the applicant or registrant has a justifiable excuse for his inability to submit such material or to make it available upon request of the Agency (such as fire, flood, or some other cause that is not the result of the applicant's or registrant's negligence nor that of his agent); or

(C) Whether the material requested is relevant to or necessary for an evaluation of the health and environmental effects of the pesticide with which the material is associated.

(ii) If the petitioner fails to sustain his affirmative burden of proof, the Administrator may issue a final order of denial of the application, a final order of cancellation of the registration, or other action, as appropriate.

(e) *Archives; identification, storage, and retrieval.*—(1) *Archives.* The applicant or registrant shall be responsible for the retention of the raw data, specimens, and samples of test substances in his own archives or those of a designated agent, and shall designate an individual whom the Agency may contact directly, with respect to any requests or questions pertaining to the archives or the material required to be retained by this section.

(2) *Identification.* All raw data, specimens, and samples of test substances shall be clearly identified in a way which assures that the material will not be confused with any material from other tests.

(3) *Storage conditions.* The raw data, specimens, and samples of test substances shall be stored under conditions which will minimize deterioration.

(4) *Retrieval.* The applicant or registrant (or designated agent) shall maintain an indexing and identification system which permits him to locate and retrieve the materials expeditiously.

§ 163.40–6 Disclaimer for named products and devices.

Mention of specific products and devices in these guidelines, including the appendices, does not imply their endorsement by the Agency in preference to other similar products and devices capable of performing the same intended functions.

Subpart D—Chemistry Requirements

§ 163.60–1 Overview.

(a) The Product Chemistry requirements, contained in section series 163.61–, emphasize two areas: defining and proving the precise content of pesticide products, and delineating up to 19 physical and chemical characteristics of these products. The description of product content must address not only the active ingredients, but also impurities and intentionally added inert ingredients. The Agency requires data on manufacturing process, analytical methods, and theoretical expectation of chemical presence to substantiate the descriptions. In addition, the applicant must certify limits on all intentionally added ingredients and on certain impurities. Physical and chemical data are required for evaluation of safety (e.g., flammability), usefulness (e.g., stability), potential environmental impact (e.g., octanol/water partition coefficient), and general identification (e.g., melting point).

(b) Data from studies on environmental chemistry (section series 163.62–) are evaluated to determine the nature and distribution of pesticide residues in the environment. The guidelines generally require data from laboratory and field tests to evaluate aspects of the following processes: physicochemical degradation, metabolism, mobility, field dissipation, and accumulation. Data are required from tests in water, air, and soils under various conditions, and from tests with microorganisms and fish. Much of the information is used in conjunction with fish and wildlife toxicity data to assess potential environmental hazards of pesticides to these organisms.

(c) Special chemistry requirements include a brief discussion of tolerance requirements for pesticides used on food or feed (section series 163.63–), and requirements for residue data involving pesticides used on tobacco (section series 163.64–).

Product Chemistry

§ 163.61–1 Definitions.

Terms used in subpart D shall have the meaning set forth in FIFRA, at § 162.3 of this chapter, and § 163.40–2. In addition:

(a) The term "impurity" means any ingredient other than an active ingredient or intentionally added inert ingredient, and includes impurities, contaminants, reaction products, and degradation products.

(b) The term "intentionally added inert ingredient" means any inert ingredient which is intentionally added to the technical grade of the active ingredient, or to the manufacturing-use product.

(c) The term "integrated formulation system" means any process for manufacturing a formulated product by use of any substance which contains an active ingredient which: (1) is not a registered manufacturing-use product, or (2) was produced or acquired by the formulator in a manner that does not permit the Administrator to inspect the substance, under section 9(a) of FIFRA, prior to use of the substance in the process.

§ 163.61–2 Scope of requirements.

(a) *Location of requirements.* The following list of paragraphs and sections of product chemistry indicates the information or materials required to support the registration of manufacturing-use products or formulated products:

Sec.
163.61-3(a) Product identity.
163.61-3(c) Disclosure of ingredients.
163.61-4 Description of manufacturing process.
163.61-5 Discussion of unintentional ingredients.
163.61-6(a) Declaration of limits.
163.61-6(b) Certification of limits.
163.61-7(a) Analytical methods.
163.61-7(b)(1) Analytical results.
163.61-7(b)(2) Spectral data.
163.61-8 Physical and chemical properties.
163.61-9 Submittal of samples.

(b) *Technical grade of the active ingredient.* In addition to the data requirements identified in paragraph (a) of this section, the following sections establish the requirements for information on the technical grade of each active ingredient which must be submitted in support of the registration of each manufacturing-use product

and each formulated product that contains the active ingredient:

Sec.
163.61-3(b) Identification.
163.61-3(c) Composition.
163.61-7 (a)(3) and (b)(1) Analytical methods and results.
163.61-7(b)(2) Spectral data.
163.61-8 (a), (b), (c)(1)-(11) Physicial and chemical properties.
163.61-9 Submittal of samples.

(c) *Deferred submittal of certain data.* Some of the information required in §§ 163.61-3 through 163.61-8 cannot be supplied until after commercial chemical production has commenced. To the extent possible, the applicant shall supply information at the time application for registration is made. Within 6 months after commercial production has commenced, the applicant shall satisfy the data requirements contained in §§ 163.61-3 through 163.61-8 which could not be satisfied at the time of registration. The applicant shall supply a schedule for satisfying these data requirements with his registration application.

(d) *Nature of data submittals.* The applicant is referred to § 163.40-1(1) of subpart B and § 162.9 of this chapter for procedures and regulations governing data submission and reference to data developed by others. In addition, for data on inert ingredients, an applicant may request the basic manufacturer of an inert ingredient to submit information directly to the Agency on his behalf. To facilitate Agency recognition of the relationship between information submitted separately and the application, the applicant must make clear and complete reference to such information in his application, and he must assure that the basic manufacturer clearly designates on his submittal the appropriate names, numbers, and companies for which the information is supplied.

(e) *Explanations for excluding certain data.* Some of the information required on §§ 163.61-3 through 163.61-8 might prove to be extremely difficult to develop, especially with respect to certain highly complex, naturally occurring organic or inorganic ingredients such as corncobs and clays. In some cases, the applicant may know of no way to even attempt to develop such data, and in other cases he may find that one or more plausible means to develop the data are in fact incapable of producing the data. In either case, the applicant shall demonstrate that all reasonable means to secure the information has been considered and attempted. If the Agency determines that such a demonstration is not adequate, it may deny registration of the product.

§ 163.61-3 Product identity and disclosure of ingredients.

(a) Each manufacturing-use product and each formulated product shall be identified in the registration application by: (1) The product name; (2) the trade name(s), (if different); and (3) the company code number(s) (optional).

(b) The technical grade of each active ingredient in a manufacturing-use product or formulated product shall be identified by: (1) Product name (if any); (2) "technical grade of (name of the active ingredient)" (for example, "technical grade of parathion"); and (3) a statement of formula identifying each reasonably identifiable substance in the technical chemical, and listing the presence of each as a percentage of the weight or as ppm (mg/kg) of the technical chemical used in the product.

(c) An application for registration of a manufacturing-use product or a formulated product shall contain the following information (if available) on each substance which is known to be present or which might reasonably be identifiable as present in the pesticide product, including active ingredients, intentionally added inert ingredients, impurities, and those reaction products and degradation products known or theorized to be formed in the pesticide product during its manufacture or during its marketable life:

(1) The chemical name from the Chemical Abstracts 1972-1976 Index of Nomenclature, or other well-defined name;

(2) The chemical abstracts (CAS) registry number;

(3) The product name, the trade name, and the common name (if established);

(4) The experimental or internal code number;

(5) For each active ingredient, the molecular (empirical) formula and the weight or the molecular weight range; and

(6) For each organic ingredient, the structural formula.

§ 163.61-4 Description of manufacturing process.

Each application for registration of a manufacturing-use product or a formulated product shall include the following information:

(a) *Composition of starting and intermediate materials.* Each starting and intermediate material used in producing a product shall be listed and identified. Identification of each material shall include:

(1) If the material is a manufacturing-use product, reference to the information on that manufacturing-use product, submitted in accordance with § 163.61-3(c); or

(2) If the material is not a manufacturing-use product, the identity and the percent of composition by weight of each substance present in the material, the expected variation in composition (to the extent that this information is reasonably ascertainable), and an explanation of how this information was derived.

(b) *Basic manufacturing process.* The following information on the basic manufacturing process of the product shall be submitted: the order in which starting materials and intermediate materials are added, and their amounts; the equations of chemical reactions; the equipment; the process solvents; the reactive conditions; a flowsheet of the process, and any purification steps that may be used; quality control measures; an indication of whether a batch or continuous process is used; and a description of materials used to package the product. The information need only include those factors which affect the composition of the product and its certified limits or ingredients.

§ 163.61-5 Discussion on formation of unintentional ingredients.

An application for registration of a manufacturing-use product or a formulated product shall contain a theoretical discussion concerning the formation of each substance, aside from active ingredients and intentionally added inert ingredients, that might reasonably be identifiable as present in the pesticide product. Such substances shall include: impurities introduced from manufacture and from ingredients, equipment, packaging, and other sources; and products of side reactions, reactions between ingredients, and of the degradation of ingredients and impurities. The theoretical discussion must address any chemical reactions that might reasonably result in the formation of these substances. The discussion shall take into account the manufacturing process, composition of starting and intermediate materials, processing variables, any purification or quality control methods used, and any other relevant factors.

§ 163.61-6 Declaration and certification of ingredient limits.

An application for registration of a manufacturing-use product or a formulated product shall contain the following:

(a) *Declaration of limits.* The applicant shall list the upper and lower limit established for each active and intentionally added inert ingredient, and the upper limit for each impurity, reaction product, and degradation product, which is known to be present or which might reasonably be identifiable as present in the product. The limits shall be stated as percentages of the total product, calculated by weight or as ppm (mg/kg).

(b) *Certification of limits.* (1) The applicant shall certify to the Agency that each upper and lower limit established in accordance with paragraph (a) of this section will be maintained

for all quantities of the product sold or distributed in commerce. Certified limits are used in two ways. First: The Agency may sample any product distributed in commerce, and the sample's failure to conform to the certified limits may be used as evidence of violation of section 12(a)(1)(C) of FIFRA. Second: The Agency will consider the certified limits in making the determinations required by section 3(c)(5) of FIFRA or in taking any other regulatory action. In stating maximum limits, the applicant should bear in mind that he must also establish that such limits will not permit use(s) of the product resulting in residues exceeding the tolerance for the chemical in food or feed. (See § 163.63-1.) The applicant should also take into account those maximum limits necessary for rapidly degrading ingredients to assure a proposed strength of the product for its stated life.

(2) For each certified limit, the application must contain an explanation of how the limit was established. First: The applicant shall show how much of the substance is present (or is likely to be present) in a sample of the product. This could be done by use of quantitative analysis of one or more of the samples, by calculations based on the amounts of various ingredients introduced in the manufacturing process, or by other means. The explanation should state the means used, and what is known about its accuracy and sensitivity. Second: When the applicant applies some factor to account for variations which might predictably occur in the amount of the substance present in any of the various samples of the marketed product, he must state what this factor is.

(3) The applicant may petition to amend his registration to certify new limits.

(4) The certification of limits shall apply until an expiration date specified on the product label, or if no such date is specified, for the life of the product.

§ 163.61-7 Product analytical methods and data.

(a) *Analytical methods.* (1) *General requirements.* Applicants shall provide descriptions of analytical methods (or references to commonly recognized analytical techniques in published scientific literature) for detecting and measuring the concentration of ingredients or impurities as indicated in paragraphs (a) (2), (3), and (4) of this section. Each method shall be described in a detailed and step-by-step manner so that it can be used properly by an analyst who may not be familiar with it. The methods should be as simple as possible and adaptable to routine chemistry laboratory use. They should include extraction procedures, qualitative confirmatory methods, and validation data, including background (or blank) values and recovery values. The applicant must supply his assessment of the sensitivity or limit of detection in each of the methods.

(2) *Manufacturing-use products.* An application for registration of a manufacturing-use product shall contain an analytical method (or reference to a method) for detecting and measuring each active ingredient, and each identifiable impurity that is present in the product in quantities of 0.01 percent (of the weight of the product) or more.

(3) *Formulated products produced by integrated formulation systems.* An application for registration of a formulated product produced by an integrated formulation system shall contain an analytical method (or reference to a method) for detecting and measuring each active ingredient, and each identifiable impurity (associated with manufacturing of the technical grade of the active ingredient) present in the formulated product in quantities of 0.01 percent (of the weight of the formulated product) or more.

(4) *All other formulated products.* An application for registration of a formulated product produced by any system other than an integrated formulation system shall contain an analytical method (or reference to a method) for detecting and measuring each active ingredient in the product.

(5) *Methods on other ingredients and impurities.* The Agency may, at any time, require submittal of analytical methodology for detecting and measuring any ingredient or impurity not named in paragraphs (a) (2), (3), and (4) of this section, such as intentionally-added inert ingredients, and identifiable impurities occurring at less than 0.01 percent of the weight of the product technical chemical.

(b) *Analytical results.* (1) *Data from submitted methods.* For each method supplied to the Agency as required by paragraph (a) of this section, an application for registration shall contain corresponding analytical results produced from the utilization of the method on five or more samples of the product. Each sample must be typical of the product produced during the manufacturing process described according to § 163.61-4. If the product is in commercial production, the samples shall represent commercial production. If produced in a batch process, the samples shall be taken from five or more different batches of the product. If produced by a continuous process, each of the five or more samples shall be taken at intervals that would represent sufficient spacing to provide different representative aliquots. If the product is not in full commercial production, aliquots may be taken in such a manner as to provide reasonable representation of the product (taking into account the known production variables). In such a case, the applicant may find it necessary to amend his registration later to certify new limits, in accordance with § 163.61-6(b)(3).

(2) *Identifying spectral data.* If requested by the Agency, identifying spectral data shall be submitted on any individual substance listed in the statement of composition in accordance with § 163.61-3(b) and (c), for any formulated product, manufacturing-use product, or technical chemical. Spectral analyses which may be required include infrared, nuclear magnetic resonance, ultraviolet, and mass spectra.

§ 163.61-8 Physical and chemical properties.

(a) *When required.* Data on the physical and chemical properties of the technical grade of each active ingredient present in the product are required to support the registration of each manufacturing-use product and each formulated product. In addition, data on the physical and chemical properties of the manufacturing-use product are required to support the registration of a manufacturing-use product, and data on the physical and chemical properties of the formulated product are required to support the registration of the formulated product.

(b) *Substance tested.* Table 1 summarizes those physical and chemical properties which must be determined by testing the technical chemical, and manufacturing-use product, and the formulated product.

(c) *Conduct of test.* The paragraphs which follow describe the conditions under which the physical and chemical properties must be determined. The appendix to this section contains references to acceptable methods for making these determinations. The applicant shall submit his own statistical evaluations of precision of the measurements (e.g., standard deviations or confidence intervals) where appropriate.

TABLE 1.—*Summary of physical and chemical properties data requirements in § 163.61-8*

Property	Test substance		
	Technical chemical	Manufacturing-use product	Formulated product
1. Color	X		X
2. Odor	X		X
3. Melting point	X		

TABLE 1.—*Summary of physical and chemical properties data requirements in § 163.61-8*

Property	Test substance		
	Technical chemical	Manufacturing-use product	Formulated product
4. Solubility	X	.....	.....
5. Stability	X	.....	.....
6. Octanol/water partition coefficient	X	.....	.....
7. Physical state	X	X	X
8. Density or specific gravity	X	X	X
9. Boiling point	X	X	X
10. Vapor pressure	X	X	X
11. pH	X	X	X
12. Storage stability	.....	X	X
13. Flammability	.....	X	X
14. Oxidizing or reducing action	.....	X	X
15. Explosiveness	.....	X	X
16. Miscibility	.....	X	X
17. Viscosity	.....	X	X
18. Corrosion characteristics	.....	X	X
19. Dielectric breakdown voltage	.....	.....	X

(1) *Color.* The color or lack of it, of the technical chemical and the formulated product based on visual inspection at room temperature, is required. Any intentional coloration or discoloration, in accordance with §162.13 of this chapter, must be reported together with the name and percent of coloring or discoloring agent and the color in terms of the Munsell color system. The applicant is referred to the appendix for the source of information on this color system.

(2) *Odor.* The characteristic odor of the technical chemical and the formulated product at room temperature is required unless there is an inhalation hazard. The odor, if any, should be reported as: "characteristic of aromatic compounds", "garlic-like", "characteristic of sulfur-containing compounds" and similar descriptive terms.

(3) *Melting point.* A melting point or a melting range in ° C is required for a solid technical chemical, unless the technical chemical sublimes of decomposes.

(4) *Solubility.* Solubility in g/100 ml of solvent at 20° C (68° F) is required for the technical chemical. If the chemical is sparingly soluble, its solubility may be reported in other terms such as ppm (mg/kg). The solubility is to be determined for representative polar and nonpolar solvents commonly used for pesticides, and especially those solvents incorporated in the applicant's product. The solubility in distilled water must also be determined.

(5) *Stability.* The applicant is required to submit information on the stability of the technical chemical. The Agency will evaluate this information to determine if the active ingredient decomposes with accompanying change in potential hazard. Some of the considerations relative to stability of the technical chemical include: sensitivity to acids, bases, and moisture; sensitivity to metal ions or to metal; stability at normal and elevated temperatures; and sensitivity to light (ultraviolet radiation or sunlight). Any decomposition observed in melting point or boiling point determinations should also be reported along with the temperature at which decomposition occurs.

(6) *Octanol/water partition coefficient.* A determination of the octanol/water artition coefficient (K) at 20° C (68° F) is required for all nonpolar organic chemicals in technical or purer form. The method suggested in the Appendix should be used for this purpose. Should solubility of the chemical be less than 1000 ppm, then the concentrations used are to be equivalent to the solubility and to half the solubility of the chemical in octanol. Data on the partition coefficient are qualitatively predictive of bioaccumulative potential and useful to the environmental impact evaluation of the registration application.

(7) *Physical state.* The physical state of the technical chemical, manufacturing-use product, and formulated product, based on a visual inspection at 68° F (20° C), is required. Conventional terms such as "solid", "granular", "semi-solid", "mixture of liquid and solid", "volatile liquid", "powder", "gas", and similar descriptive terms should be used when applicable.

(8) *Density or specific gravity.* For a technical chemical, manufacturing-use product, and formulated product in the solid state, the density (in g/ml) at 20° C (68° F) is required. For a liquid, the specific gravity at 20° C referred to water at 20° C is required.

(9) *Boiling point.* A boiling point or boiling range in °C is required for a liquid technical chemical, manufacturing-use product, and formulated product unless the characteristics of the chemical or product make this determination impossible. The determination may be made at 760 mm Hg or at a reduced pressure, when appropriate, in consideration of the inherent properties of the chemical.

(10) *Vapor pressure.* The vapor pressure in mm Hg, Torr, or psig, where appropriate, at 20° C (68° F) is required for the technical chemical, and at 40° C for the manufacturing-use product and formulated product. The Agency will evaluate these data for problems which may be encountered in packaging and in the intended use.

(11) *pH.* Determinations of pH are required for any technical chemical, manufacturing-use product, or formulated product which can be diluted or dispersed with water. A determination is to be made in accordance with the method described in the Appendix. The technical chemical or product should be diluted or dispersed with distilled water. The Agency will evaluate these data for compatibility of mixtures and for hazards arising from high corrosivity.

(12) *Storage stability.* The pesticide product as commercially packaged must be stable under storage conditions stated on the label (or in the absence of such instructions, under conditions typical for storage of the product). The Agency therefore requires data on the storage stability of a manufacturing-use product and a formulated product in its commercial package for a period of at least one year at a temperature of 20° C (68° C), and, when the commercial package is permeable, at a relative humidity of 50 percent. Data derived from an accelerated aging test of at least 30 days duration at 50° C (122° F) are acceptable to support the product's conditional registration. If the percentage of any active ingredient decreases by more than 10 percent of its original value in either study, then the provisions of § 162.10(g)(6) of this chapter concerning label warnings of deterioration (expiration date) will apply. The applicant must specify an expiration date on the label which would permit the product to be used only while its composition still conforms to the limits certified under § 163.61-6(b). The applicant must determine if any deterioration or degradation products develop in the product and must submit this information as part of the statement on composition required by § 163.61-3(c). Any unusual physical changes, such as phase separation in the product during aging, shall also be reported.

(13) *Flammability.* A determination of the flash point is required for all combustible manufacturing-use products and formulated products. When the product is intended for packaging under pressure, the flash point is to be determined with the propellent-free portion of the formulation before it is packaged. The flame extension or flame projection is also to be determined for a formulated product while in the package under pressure. An evaluation of flammability based on these determinations will indicate whether or not warning statements required by § 162.10(h)(2)(iii) (A) and (B) of this chapter are needed. Caution is advised in conducting these tests.

(i) *Flash point.* The flash point in °F (°C) must be determined by a closed cup test method. The Tagliabue (TAG) Closed Tester should be used

for mobile liquids (those having a viscosity higher than that of ethylene glycol). The Pensky-Martens Closed Tester should be used for all other liquids. Setaflash ® or other recognized closed cup testers are acceptable alternatives.

(ii) *Flame extension.* The flame extension or flame projection is to be measured to the nearest inch (2.5 cm) by the method suggested in the Appendix.

(14) *Oxidizing or reducing action.* Information is required on the tendency of a manufacturing-use product or a formulated product to act as an oxidizing or reducing agent as a result of the presence in the formulation of a highly reactive ingredient. The Agency seeks to avoid chemical combinations that are hazardous in the use or storage of the pesticide, i.e., combinations which may result in the generation of noxious fumes, ignition, or even explosion. No method fically designed to test this tendency has been provided in the Appendix, and the necessary information must be gathered from a knowledge of chemistry or other experience.

(15) *Explosiveness.* Information is required on the impact explosion characteristics, if any, of a manufacturing-use product and formulated product resulting from the presence of a potentically explosive ingredient in the product. The product should be subjected to the standardized sensitivity test of the Bureau of Explosives, Association of American Railroads. Caution is advised in conducting the test.

(16) *Miscibility.* For liquid products which are intended primarily for dispersal with water, but which may also be diluted with petroleum solvents, information is required to demonstrate the ability of the product to physically continue to function when used in accordance with dilution and use instructions on the proposal label. These data are intended to show suitability for application after dilution with the various petroleum solvents specified on the label. The determination is to be made at 20° C (68° F) by first diluting and mixing the product with petroleum solvents whose composition and rate of dilution reflect those on the label, then examining for separation after standing for 30 minutes.

(17) *Viscosity.* Viscosity determinations are required for each liquid manufacturing-use product and liquid formulated product, and will be used by the Agency to anticipate problems in dilution. A determination in terms of poises or stokes at 20° C (68° F) by any of several techniques described under the method listed in the Appendix is required.

(18) *Corrosion characteristics.* Data on the effect, if any, of the manufacturing-use product and formulated product on its paekage are required for evaluating potential for leakage. The requirement is an extension of the stability requirements set forth in paragraph (c)(5) of this section, and applies to those formulations whichcome into contact with metal when commercially packaged in metal containers. Accelerated corrosion tests of 30 days duration at 50° C (122° F) using immersed specimens of the metal(s) of the container are acceptable. The appendix contains a suggested test procedure.

(19) *Dielectric breakdown voltage.* A dielectric breakdown voltage measurement is required only for those nonconducting liquid formulated products specifically intedned for use in or around electrical equipment. The Agency will evaluate this information to determine if there is a likelihood of electrical hazard to the user of the formulated product. The determination is to be made at 20° C (68° F) according to the method recommended in the Appendix.

(d) *Discontinued requirements.* Information on the percentage free alkali, the dissociation constant, and flammability derived from the drum test, is no longer required.

§ 163.61-9 Submittal of samples.

When required by the Agency, the applicant shall submit a sample of the technical chemical, manufacturing-use product, or formulated product conforming to the limits certified under § 163.61-6(b), to:

Supervisory Chemist, Quality Assurance Analytical Chemistry Section, Chemical and Biological Investigations Branch, Technical Services Division, OPP, U.S. Environmental Protection Agency, Room 101, Building 306, ARC-East, Beltsville, Md. 20705.

APPENDIX TO PRODUCT CHEMISTRY

The applicant is referred to the following published sources for suggested test methods which are considered acceptable or adaptable for compiling the product chemistry data required for registration: CIPAC Handbook, Volume 1, on "Analysis of Technical and Formulated Pesticides", compiled by R. deB. Ashworth, J. Henriet, and J. F. Lovett, edited by G. R. Law, and published by Collaborative International Pesticides Analytical Council Limited, Plant Pathology Laboratory, Hatching Green, Harpenden, Hertfordshire, England (1970); and "1974 Annual Book of ASTM Standards", published by the American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pa. 19103.

The test methods for physical and chemical properties are found in the above-named published sources as follows:

Boiling point: ASTM D1078-70.
Color: ASTM D1535-68. Information on the Munsell color system may be obtained from Munsell Color, MacBeth Color and Photometry, Div. of Kollmorgen Corp., 2441 N. Calvert St., Baltimore, Md. 21218.
Corrosion characteristics: ASTM G31-72.
Density or specific gravity: CIPAC MT 3, ASTM D941-55, ASTM D1480-62, ASTM D1481-62, and ASTM D1217-54.
Dielectric breakdown voltage: ASTM D877-67.
Flame extension or projection: ASTM D3065-72.
Flash point: CIPAC MT 12, ASTM D56-70, ASTM, D93-73, and ASTM D3278-73.
Melting point: CIPAC MT 2.
Miscibility: CIPAC MT 23.
Octanol/water partition coefficient: 40 FR 26880 (June 25, 1975) *pH:* ASTM E70-74.
Solubility: CIPAC MT 4, MT 5, MT 6, MT 7, MT 8, MT 9, MT 10, MT 11, MT 27, and 40 FR 26879 (June 25, 1975).
Stability: CIPAC MT 39, MT 41, MT 46, and MT 55.
Storage stability: CIPAC MT 46.
Vapor pressure: 40 FR 26889 (June 25, 1975), ASTM D3074-72.
Viscosity: CIPAC MT 22.

The applicant is referred to the following published sources for additional information.

1. *Physical and chemical properties.*

Weissberger, A., ed., 1959-70. Technique of organic chemistry. Interscience Publishers, New York, N.Y.

2. *Analytical methods for the technical chemical and formulated products.*

Bontoyan, W. R., ed. 1975. Manual of chemical methods for pesticides and devices. U.S. Environmental Protection Agency, 401 M Street SW., Washington, D.C. 20460.

Raw, G. R., ed. 1970. CIPAC Handbook, Vol. 1. Collaborative International Pesticides Analytical Council Limited, Plant Pathology Laboratory, Hatching Green, Harpenden, Hertfordshire, England.

Welcher, F. J., ed. 1963. Standard methods of chemical analysis. 6th ed., D. Van Noostrand, Princeton, N.J.

Zweig, G. and J. Sherma, eds. 1963-1976. Analytical methods for pesticides and plant growth regulators. Continuing series; Academic Press, New York, N.Y.

ENVIRONMENTAL CHEMISTRY

§ 163.62-1 Purpose: hazard assessment.

(a) *Relationship to hazard assessment.* The introduction of pesticides to the environment can result in possible adverse effects on man and other nontarget species. The potential adverse effects are complex, interrelated, and engender complex hazard assessment. Required data on environmental chemistry characteristics used to assess hazards are categorized in the Guidelines as *physicochemical degradation, metabolism, mobility, field dissipation,* and *accumulation.* Environmental hazards and their consequences described by specific environmental chemistry studies within these categories are assessed according to: (1) Direct consequences to man via his exposure to pesticide residues remaining after application either upon his reentering treated areas or through his ingestion of contaminated rotational crops; (2) indirect consequences to man from the presence of widely distributed and persistent pesticide re-

sidues in the environment which may result in loss of usable land, water, and wildlife resources; and (3) potential environmental exposure of other nontarget organisms (including fish and wildlife) to pesticide residues that may either be taken up and accumulated in the food web or result in loss of habitat.

(b) *Direct vs. indirect exposure patterns.* The complexity of the environmental hazard assessment and the nature of the supporting environmental chemistry data requirements are dependent not only on the extent and duration of exposure but also in some instances on the route of exposure. For example, pesticides may be applied directly to the aquatic environment (e.g., mosquito larvicides and aquatic herbicides) or their residues may be discharged into the aquatic environment following either interception of a pesticide by sewage treatment systems or use of pesticides for pulp and paper mill, cooling tower, and waste water treatments. In the examples cited, pesticide residues impacting on the aquatic environment via the direct route of exposure can differ both qualitatively and quantitatively from those generated via the indirect route of exposure due to the residence time of the pesticide in the treatment process.

§ 163.62-2 Definitions.

Terms used in the sections on environmental chemistry shall have the meanings set forth in FIFRA, at §162.3 of this chapter, and at §163.40-2 and §163.60-2. In addition:

(a) The term "material balance" means a quantitative accountability of a chemical, including both identified and unidentified products, throughout a test with a defined system into which the chemical has been introduced.

§ 163.62-3 Scope of requirements.

(a) *Conditions under which specific date or tests are required.*—(1) *Specific Uses.* Sections 163.62-6 through 163.62-13 specify the data required to support registration of a formulated product and the data required to support registration of a manufacturing-use product. Table 2 (Summary of environmental chemistry data requirements by intended use pattern) summarizes these data requirements. Other data will be required as necessary to evaluate whether a pesticide as used causes unreasonable adverse effects on man or the environment.

TABLE 2. SUMMARY OF ENVIRONMENTAL CHEMISTRY DATA REQUIREMENTS BY INTENDED USE PATTERN

DATA REQUIREMENTS[1]	TERRESTRIAL USES					AQUATIC USES		TERRESTRIAL/AQUATIC USES	AQUATIC IMPACT USES		
	DOMESTIC OUTDOOR	GREENHOUSE	NON-CROP	TREE FRUIT-NUT CROP	FIELD-VEG. CROP	AQUATIC FOOD CROP	AQUATIC NON-CROP	FOREST	DIRECT DISCHARGE	INDIRECT DISCHARGE	WASTEWATER TREATMENT
PHYSICO CHEMICAL DEGRADATION											
HYDROLYSIS	x	x	x	x	x	x	x	x	x	x	x
PHOTODEGRADATION			x	x	x	x	x	x	x		
METABOLISM											
AEROBIC SOIL	x	x	x	x	x			x			
ANAEROBIC SOIL					x						
ANAEROBIC AQUATIC						x	x	x	x		
AEROBIC AQUATIC						x	x		x		
EFFECTS OF MICROBES ON PESTICIDES			x	x	x	x	x	x	x		
EFFECTS OF PESTICIDES ON MICROBES			x	x	x	x	x	x[a]	x		
ACTIVATED SLUDGE										x	x
MOBILITY											
LEACHING			x	x	x			x[b]			
VOLATILITY		x									
ADSORPTION	x	x	x	x	x	x	x	x	x		
WATER DISPERSAL						x	x		x		
FIELD DISSIPATION											
SOIL	x		x	x	x	x		x			
WATER						x	x	x[c]	x		
ECOSYSTEM (x[d] COMBINED STUDY WITH x[a], x[b], x[c])								x[d]			
ACCUMULATION											
ROTATIONAL CROP					x	x					
IRRIGATED CROP						x	x				
FISH			x	x	x	x	x	x	x		
SPECIAL STUDY							x				

[1] DATA REQUIREMENTS CITED IN §163.62-10(c)(3), (f) AND (g); §163.62-11(e); §163.62-12; AND §163.62-13 ARE NOT INCLUDED IN THIS TABLE

FEDERAL REGISTER, VOL. 43, NO. 132—MONDAY, JULY 10, 1978

(2) *Unspecified uses.* The Agency recognizes that some uses are not easily categorized. Data requirements for pesticides employed for these uses must be determined on an individual basis following consultation with the Agency.

(b) *Appendix references.* The appendix consists of referenced examples of published literature containing acceptable procedures and supplemental background or ancillary material which may be consulted in developing acceptable protocols. The appendix consists of a series of items keyed by subject heading to the appropriate sections of this subpart.

§ 163.62-4 Basic standards for testing.

(a) *Personnel.* All testing and evaluation must be done under the direction of personnel who have the education, training, and experience to perform the testing and evaluation in accordance with sound scientific experimental procedures. The Agency may request a resume of the qualifications of personnel who have performed, supervised, reviewed, or evaluated the testing.

(b) *Test controls.* The Agency recognizes the necessity of adequate experimental control values in the data generated by laboratory and field experiments, and requires their submission. However, due to the wide diversity of pesticide properties, use patterns, and organisms exposed in the field environment, specific requirements for reporting control values (as to source, sampling regime, and total number submitted) will depend upon the complexity and variability of the environment in which the test is to be conducted.

(c) *Test substance.* (1) The data required by §§163.62-7 through 163.62-13 shall be derived from tests conducted with the following test substances:

(i) Laboratory or greenhouse studies which specify use of radioisotopic analytical techniques shall be conducted with the analytical grade of each active ingredient in the product.

(ii) Laboratory or greenhouse studies which specify use of non-radioisotopic analytical techniques shall be conducted with the technical grade of each active ingredient in the product. The technical chemical is commonly the same substance as the manufacturing-use product for which registration is sought or which is used to produce the formulated pesticide product for which registration is sought. In this case, a sample of the manufacturing-use product shall be tested. Where this is not the case, the tests shall be conducted with the technical grade of the active ingredient which is used to produce the manufacturing-use or formulated pesticide product.

(iii) Except as provided in § 163.62-10(f), all outdoor or field studies shall be conducted with a formulated product representative of one predominant type of formulated product that would be made from each manufacturing-use product.

(iv) In addition to or in lieu of testing otherwise required by this subpart, the Administrator may require testing to be conducted with:

(A) An analytically pure grade of an active ingredient;

(B) The technical grade of an active ingredient;

(C) An intentionally added inert ingredient of a pesticide formulation;

(D) A contaminant or impurity of an active or inert ingredient;

(E) A plant or animal metabolite or degradation product of an active or inert ingredient;

(F) The pesticide formulation; or

(G) Any additional substance which could act as a synergist to the product for which registration is sought.

(2) The test substance used shall be within the limits, if any, certified in accordance with § 163.61-6(b). The composition of the test substance shall be determined, including the name and quantities of known contaminants and impurities as far as is technically feasible. The determination shall also include quantities of unknown materials (if any), so that 100 percent of the test sample is accounted for.

(3) The lot and sample numbers of the test substance shall be determined and recorded.

(4) The test substance should be stored under conditions that maintain its identity, strength, quality, purity, and stability.

(d) *Field studies.* Field studies shall be conducted in areas representative of where the pesticide is intended to be used.

§ 163.62-5 Reporting of data.

(a) *General.* Standardization of data reporting and submission of a separate volume of environmental chemistry data will expedite the review process. Multipurpose data must be referenced to specific pages in other volumes or duplicated and submitted in each appropriate volume.

(b) *General reporting requirements.* (1) *Test report.* The test report shall include information necessary to provide a complete and accurate description of test procedures and evaluation of the test results. The test report shall include a summary of the data, an analysis of the data, and a statement of conclusions drawn from the analysis. The report shall include any information required by §§ 163.62-6 through 163.62-13 and the following.

(2) *Test method.* The statement of test method used shall include a full description of the experimental design and procedures; the site(s) or location(s), length, and actual dates of the study; the name and address of the laboratory or institution performing the test; and names of principal investigators.

(3) *Test substance.* (i) Identification of the test substance including chemical name, molecular structure, and qualitative and quantitative description of its chemical composition, including the results of the analysis conducted in accordance with § 163.61-7(b)(1).

(ii) Manufacturer and lot and sample numbers of the test substance.

(iii) Properties of the substance tested, such as physical state, pH, and stability.

(4) *Units of measurement and calculations.* (i) Reporting units must be in the metric system, but the English system may be used, in addition. In no instance should the systems be mixed (e.g. kilograms/acre).

(ii) Residue data must be reported as follows: in soil as ppm or as mg/kg; in water, as mg/1 or ppm; in tissue, as mg/kg or ppm.

(iii) Volatility data are to be reported in g/ha/day, air concentration in ug (ng)/m^3, vapor in ug/1, and vapor pressure in mm Hg.

(iv) Mathematical equations used and calculations based on the equations must be submitted with each study. When rates of formation and decline are reported in any test, data should be expressed both as amounts and corresponding percentages. Appropriate rate constants should be reported in conjunction with rate data. Tabular data are required. Graphs for decline curves and soil sorption are required.

(5) *Data presentation.* (i) A list of identifiable residues by industrial code number, chemical or company names, chemical structures, and conditions where they are found (e.g., photodegradation, fungal decomposition, hydrolysis) is required.

(ii) Characterization of soils must be reported, including texture (percent sand, silt, and clay), percent organic matter, pH, cation exchange capacity, and bulk density. Characteristics of water must be reported, including, but not limited to, pH, temperature, and oxygen content.

(iii) Records such as gas chromatograms, photographs, and diagrams of thin-layer chromatography (TLC) plates, infrared spectra, and other similar records must be submitted to support recovery data on analytical methods. Similarly, representative raw data must be submitted for maximal and minimal values of analyses from metabolism, accumulation, and field dissipation studies. Submitted field report data, including gas and thin-layer chromatograms, must be reproduced for rotational crop analyses that show residues.

(iv) Design of test procedures and equipment must be described. Photographs or other reproductions of nonstandard equipment must be supplied.

(c) *Specific reporting requirements*—(1) *Photodegradation data.* Photodegradation data must be supported by incident light intensity and percent transmission. Light transmission should be reported in absorbance units and as a function of wavelength. Values for intensity are required for artificial light sources. Latitude, time of year, atmospheric cover, and other major variables which affect incident light are to be reported when natural sunlight is used.

(2) *Adsorption data.* Adsorption data must include amounts of pesticide in solution and in soil, and a calculation of the soil/solution ratio. Values of constants 1/n and K must be supplied when the Freundlich equation is used.

(3) *Field data.* Data which are to be reported from field tests include precipitation (accumulated from first application to each sampling), water table, grade (slope), and soil type. In addition, dates of planting and harvesting; application and sampling time; dates and stages of crop and pest development; application-to-harvest and application-to-sampling intervals for each treatment; and depth, weight, or volume of each sample and weights and volumes of aliquots taken for analysis must be reported. When water flow is measured, flow meters or comparable techniques are required, and flow data must be expressed in gallons per minute and liters per minute.

(4) *Microbial data.* Information on identity of organisms and media must be supplied. Organisms used as indicators must be identified by scientific name. Cultures of microorganisms obtained from collections must also be identified by collection code numbers; other sources of microorganisms must also be described. Standard maintenance and test media must be identified and described.

§ 163.62-6 Use patterns associated with data requirements.

Environmental chemistry data are required for pesticides intended for outdoor applications, including those uses with consequences extending beyond enclosed man-made structures. Each pattern of use may result in a specific identifiable potential hazard, and the environmental chemistry data are used in assessment of the hazard potential. For the purposes of this section, uses are grouped into terrestrial, aquatic, terrestrial/aquatic (forest), and aquatic impact. Categories within these use patterns are dependent on exposure to man and other non-target organisms.

(a) *Terrestrial.* This category includes, but is not limited to, domestic outdoor, greenhouse, noncrop, fruit and nut crop, and field and vegetable crop uses.

(1) *Domestic outdoor uses.* These uses are associated with the homelife but are external to dwellings. These include, but are not limited to, lawn and garden pesticide uses. Data requirements for products intended for domestic outdoor applications are: estimates of rates of degradation in water (hydrolysis), degradation and metabolism in soil (aerobic soil metabolism and field soil dissipation), and adsorption.

(2) *Greenhouse uses.* Use of products in greenhouses may present a reentry hazard. To assess this potential hazard, data on volatility and adsorption are needed as well as hydrolysis and aerobic soil metabolism data.

(3) *Non-crop uses.* These uses may result in more complex risks to nontarget organisms in the environment than the two previously described. Data requirements for products intended for these uses reflect this complexity: environmental fate is assessed by degradation, metabolism, mobility, and dissipation studies, while potential hazards associated with fate of pesticides are assessed by food web accumulation. (See the nine required studies listed in table 2 of § 163.62-3(a)(1).)

(4) *Tree fruit and nut crop uses.* Products intended for these uses must be supported by data as set forth under paragraph (a)(3) of this section.

(5) *Field and vegetable crop uses.* Products intended for these uses must be supported by data as set forth in paragraph (a)(3) of this section, as well as anaerobic soil and rotational crop residue studies.

(b) *Aquatic uses.* This category includes aquatic food crop and aquatic non-crop uses.

(1) *Aquatic food crop uses.* Products intended for these uses include, but are not limited to, crops such as rice, cranberry, taro, and watercress. The data requirements include degradation, metabolism, mobility, dissipation, and accumulation (to include analysis of residues in and phytotoxicity to rotated and irrigated crops). (See the 13 required studies listed in table 2 of § 163.62-3(a)(1).)

(2) *Aquatic non-crop uses.* Products intended for these uses include, but are not limited to, the intentional applications of pesticides directly to water to control waterborne pests in areas where food crops are not produced. Such uses employ products used as larvicides, piscicides, algicides, aquatic weed herbicides, and molluscicides. The data requirements include degradation, metabolism, mobility, dissipation, and accumulation. (See the 12 required studies listed in table 2 of § 163.62-3(a)(1).)

(c) *Aquatic impact uses.* These outdoor applications have indirect and direct consequences extending beyond enclosed manmade structures, including the effects from pulp and paper mill water treatments, and industrial cooling tower treatments. These treatments discharge pesticides either indirectly into the aquatic environment through municipal and industrial wastewater treatment facilities or directly into the aquatic environment.

(1) *Indirect discharges and wastewater treatment.* Use patterns included in this category involve those products which enter the aquatic environment but are not discharged directly into lakes, ponds, or streams, and also involve those products used directly in wastewater treatment systems (e.g., filter fly control products). Interception of pesticides by sewage treatment systems may avert or mitigate the potential contamination of the aquatic environment. Data requirements for pesticides expected to enter sewage treatment system include hydrolysis and reporting of effects on activated sludge to provide the necessary assurance that the potential hazard has been averted or mitigated by sewage treatment processes.

(2) *Direct discharges.* Use patterns included here consist of manufacturing-use products which are discharged directly into the aquatic environment. When interception by sewage treatment does not occur, a more complex hazard assessment is required. The data requirements include degradation, metabolism, mobility, dissipation, and pesticide accumulation in the food web. (See the ten studies listed in table 2 of § 163.62-3(a)(1).)

(d) *Terrestrial/aquatic (forest) uses.* These uses, including forest and reforestation sites, ecologically are transitional between the terrestrial and aquatic environments. Forest ecosystems are delicately balanced. Balance and ecological sensitivity are critical in that the forest composition may be drastically and irreversibly changed if essential environmental components (flora and fauna) are eliminated. Therefore, the complexity and sensitivity of the forest ecosystem requires a coordinated study to assess potential hazards. Data requirements include degradation, metabolism, dissipation, and accumulation. (See the 12 studies listed in table 2 of § 163.62-3(a)(1).)

§ 163.62-7 Data requirements: Physicochemical Degradation.

(a) *General.* Degradation studies are used to determine rate of decomposition and identification of pesticide residues which may adversely affect nontarget organisms. Pesticides and their degradation products may be available to nontarget organisms as residues in rotational crops and may contaminate the food web. Hydrolysis can occur in both the soil and aquatic environments, and is pH- and tempera-

ture-dependent. Photolysis is governed by intensity and wavelength of radiation and by the matrix (air, soil, or water) in which the pesticide resides.

(b) *Hydrolysis.* (1) Pesticides may enter natural water via direct application, mobility from treated areas, industrial discharge, and as a result of disposal and cleanup of containers and equipment. Hydrolysis data are required to support the registration of all manufacturing-use and formulated products. Laboratory studies are to be conducted in darkness using radioisotopic analytical techniques or nonradioisotopic techniques at different pH values (acidic, neutral, and basic) at two concentrations and two temperatures.

(2) Aliquots in duplicate should be taken at four sampling time intervals, with at least one observation made after one-half of the pesticide is hydrolyzed or 30 days, whichever is shorter. A material balance (see §163.62-2 for definition), half-life estimate, and identification of each degradation product greater than 10 percent of the pesticide must be provided. Studies utilizing distilled or deionized water are required to provide a standard. Hydrolysis in natural waters may be carried out to supplement studies in distilled water. For an acceptable procedure and supplemental information on hydrolysis studies, see appendix item 1.

(c) *Photodegradation.* Sunlight may destroy or chemically alter pesticides in soil, water, and air. Photodegradation studies in water are required to support the registration of all formulated products intended for terrestrial (except greenhouse and domestic outdoor), aquatic, terrestrial/aquatic (forest), and aquatic impact (except indirect pesticide discharges and discharges into wastewater treatment systems) uses. Studies in soil are required to support the registration of all formulated products intended for crop uses and terrestrial/aquatic (forest) uses. Studies in vapor phase are required as a part of the assessment of reentry hazard. Laboratory photodegradation studies are conducted using radioisotopic or nonradioisotopic analytical techniques at one concentration under natural or simulated (greater than 280 nm wavelength) sunlight. Such studies must provide a material balance, half-life estimate, and the identification of each photoproduct greater than 10 percent of the pesticide. Rate studies are conducted in distilled or deionized water, and sampling should continue for identification of photoproducts to half-life, or thirty days, whichever comes first. Supplemental rate and photoproduct studies may be carried out in natural water aquatic uses. Studies performed on the same soil as specified in the Aerobic soil metabolism study of §163.62-8(b) are preferred but other soil textures will be acceptable. A complete record of the intensity of incident sunlight and time of exposure must be reported if sunlight is used as the light source. Information on artificial light sources should include nature of source, intensity, wavelength distribution, and time of exposure. For acceptable procedures and supplementary information on photolysis, see appendix item 2.

§ 163.62-8 Data requirements: Metabolism.

(a) *General.* Pesticides introduced into the environment come in contact with soil and water and can be transformed via physico-chemical or metabolic means. Although physico-chemical degradation is important, metabolic transformation can be the major route in soil. Metabolic processes are important in the aquatic environment, though physico-chemical processes may predominate. Metabolism studies determine the nature and availability of pesticide residues to rotational crops and help in assessment of potential disposal and reentry hazards. Assessments of the potential for adverse effects on nontarget organisms as a result of metabolic transformation and physico-chemical degradation are similar. Metabolic transformation is soil and water is to be investigated under aerobic and anaerobic conditions. Some aspects of microbial transformation together with the effect of pesticides on microbial activities are the subject of separate metabolism studies specified in paragraphs (f) (2) and (3) of this section, and table 2 of §163.62-3(a)(1).

(b) *Aerobic soil metabolism.* This laboratory study is required to support the registration of all formulated products intended for terrestrial uses and terrestrial/aquatic (forest) uses. Rate, type and degree of metabolism of the pesticide residues are to be determined in a sandy loam, loam, silt loam, or other textured soil appropriate to the intended application sites. Where the product has broad uses, tests with 3 or more different soils are preferred. Radio-labeling in one or more positions in the pesticide molecule is required to assure adequate accounting of chemical transformations. Where radio-labeling will be of little benefit, or is difficult to achieve, nonradioisotopic analytical techniques are required. Residues comprising more than ten percent of initial application or 0.01 ppm, whichever is greater, should be identified. a material balance, including non-extractable residues, must be provided. The experimental dose rate must approximate the field application rate. Treated soil must be maintained at temperatures of 18 to 30° C at or below 75 percent of 0.33 bar moisture content. Data must be collected until a ninety percent loss of the pesticide occurs and until patterns of formation and decline of metabolic products are established. Preferred sampling times are at pretreatment, 0, 1, 2, and 7 days, 2 and 3 weeks, and 1, 2, 3, 4, 6, 9, and 12 months. The study need not be conducted for more than one year for terrestrial crop and non-crop uses, and terrestrial/aquatic (forest) uses. A study extending to two half-lives of pesticide or to 6 months duration whichever comes first, is required for pesticides employed for greenhouse uses and domestic outdoor uses. For acceptable procedures and supplemental information on soil metabolism studies, see appendix item 3.

(c) *Anaerobic soil metabolism.* This laboratory study using radioisotopic or non-radioisotopic analytical techniques is required to support the registration of all formulated products intended for field and vegetable crop uses to determine differences in rate and patterns of metabolism between aerobic and anaerobic soil conditions. However, this study need not be run if an anaerobic aquatic metabolism study in accordance with paragraph (d) of this section has been conducted. Terrestrial anaerobic soil studies should use the same soil(s) as specified in paragraph (b) of this section. Obtain an aliquot of soil at the one-month sampling interval as specified in paragraph (b) of this section, and establish anaerobic conditions by either waterlogging or purging with inert gases. Where organic content of the soil is deficient, organic amendment should be supplied within 30 days to ensure anaerobic conditions. Preferred sampling intervals are 30 and 60 days after anaerobic conditions have been established. For acceptable procedures and supplemental information on soil metabolism studies, see appendix item 3.

(d) *Anaerobic aquatic metabolism.* This laboratory study using radioisotopic analytical techniques is required to support the registration of all formulated products intended for aquatic, terrestrial/aquatic (forest), and aquatic impact uses which result in direct discharges into the aquatic environment. Anaerobic conditions are more likely to prevail where pesticides are found in aquatic environments than in strictly terrestrial environments. An anaerobic study assesses the availability of pesticide residues to irrigated crops, and to other part of the aquatic food web. Rate, type, and degree of metabolism of the pesticide are determined in water plus sediment taken from an impoundment like that found at or near proposed use sites. The preferred substrate for this laboratory study is sediment, but the use of flooded soils may be adequate. Oxygen depletion is established by flooding for 30 days prior to dosing at the approxi-

mate field rate. Where organic content of the soil is deficient, organic amendment should be supplied within 30 days to ensure anaerobic conditions. Data are to be collected until a 90 percent loss of pesticide occurs and until patterns of formation and decline of metabolites are established in water and sediment, or for 1 year, whichever comes first. Radiolabeled chemicals are to be used as specified in the aerobic soil metabolism study (paragraph (b) of this section). This anaerobic aquatic metabolism study may substitute for the anaerobic soil metabolism study [paragraph (c) of this section], but the reverse is not true. For acceptable procedures for this test, see appendix item 4.

(e) *Aerobic aquatic metabolism.* This laboratory study using radioisotopic techniques is required to support the registration of all formulated products intended for aquatic uses and aquatic impact uses which result in direct discharges into the aquatic environment. Radiolabeled chemicals are to be used as specified in the aerobic soil metabolism study (paragraph (b) of this section). To determine pesticide exposure to aerobic conditions in water during the period of dispersal throughout the aquatic environment, and to compare rates and formation of metabolites with those observed under conditions of anaerobic aquatic metabolism, pesticides must be studied in water representative of that found at or near the intended use sites and at a rate equivalent to the anticipated rate of application or discharge into the aquatic environment. These studies are to continue until decline curves have been established or for 30 days, whichever occurs first. For acceptable procedures for this test, see appendix item 5.

(f) *Microbial metabolism*—(1) *General.* Biochemical transformation may be of greater importance than physico-chemical degradation under conditions where anaerobic conditions preclude oxidation, or where photolysis is inhibited because of lack of sunlight. Microbes are among the most important groups of organisms involved in the biochemical transformation of pesticides in soil and sediments. Microbes may affect rates of degradation and formation of metabolites, and pesticides may inhibit specific segments of the microbial population important to soil fertility. Microbe-pesticide interactions may affect availability of pesticides to nontarget organisms and accumulation in the food web, or may result in loss of usable land and water resources. Data of the effects of microbes on pesticide degradation and effects of pesticides on microbes are required to support the registration of all formulated products intended for the following uses: terrestrial nonrop, tree fruit/nut crop, field/vegetable crop, aquatic food crop and nonrop, terrestrial/aquatic (forest), and direct discharge aquatic impact uses.

(2) *Effects of microbes on pesticides.* Impact of microbes on pesticide transformation is measured by laboratory comparisons of metabolic processes conducted with radioisotopic or nonradioisotopic analytical techniques under sterile and non-sterile conditions during a 30-day period. Preferred sampling intervals are 1, 3, 7, 14, 20, and 30 days, but other intervals may be appropriate. (See also the intervals specified under paragraph (b) of this section.) Acceptable oil sterilization methods are heat or high energy ionizing radiation. Attempts should be made to identify organisms responsible for pesticide degradation. Alternately, studies utilizing pure or defined and characterized mixed cultures of bacteria, algae, and/or fungi are adequate. For acceptable procedures and supplemental information on this test, see appendix items 3 and 5.

(3) *Effects of pesticides on microbes.* Data on effects of pesticides on microbes are obtained from laboratory studies employing non-radioisotopic analytical techniques to determine effects on microbial functions or microbial populations. Studies of effects on microbial functions constitute a more direct approach, and are preferred to studies of effects on populations. Some effects cannot be measured directly and population studies may be the only recourse. Studies should be conducted over a long enough period to demonstrate whether there is a temporary or long lasting effect on microbes. Data shall be supplied on oxygen consumption, carbon dioxide evolution, nitrogen cycle reactions, and measurement of enzyme activity for dehydrogenase or phosphatase. When the microbial functional approach is chosen, data on the effects on nitrogen fixation, nitrification, and degradation of cellulose, starch, and protein are required for pesticideds employed for terrestrial and aquatic uses; for pesticides employed for terrestrial/aquatic (forest) uses, a pectin degradation study is also required. As an option for terrestrial/aquatic (forest) uses, a leaf litter degradation study may be substituted for the cellulose, starch, protein, and pectin degradation studies. When the population approach is chosen, effects on pure or mixed culture populations of representative microorganisms from soil or water, or obtained from culture collections, should be recorded for terrestrial/aquatic (forest) or aquatic uses. Appropriate organisms included free-living nitrogen-fixing bacteria and blue-green algae such as *Azotobacter, Clostridium, Nostoc,* and nitrifiers such as *Nitrosomonas* and *Nitrobacter.* For cellulose, starch, pectin, protein, and similar substrates, at least one each of soil bacteria, actinomycetes, and molds such as *Bacillus, Pseudomonas, Arthrobacter, Cellulomonas, Cytophaga, Streptomyces, Penicillium, Flavobacterium, Trichoderma, Aspergillus, Chaetomium,* and *Fusarium* should be included. Animals, plant pathogens, and indicators of fecal pollution are unsuitable. For acceptable procedures for these tests, see appendix item 7.

(g) *Activated sludge metabolism.* For assessment of this protential contamination of the aquatic environment, a laboratory study of effects of pesticides on the wastewater treatment process is required to support the registration of all manufacturing-use products and all formulated products which are indirectly discharged into wastewater treatment systems or are used as treatments in wastewater treatment systems. Some pesticides discharged into these systems, if toxic to microorganisms, may disrupt the treatment process by killing the microorganisms responsible for normal wastewater treatment. Pesticides subsequently entering the affected systems are therefore not transformed by the disrupted wastewater treatment process and consequently are discharged directly into the aquatic environment. Synthetic sewage (nutrients) and the radiolabeled active ingredient should be added to activated sludge and aerated in a closed system for 23 hours, allowing the sludge to settle for 30 minutes. A liter of supernatant (effluent) should then be removed for pesticide residue analysis including a material balance. Fresh synthetic sewage and the active ingredient should then be added to the remaining sludge and the cycle repeated. Dosage should start at 0.1 ppm and increase by increments to 100 ppm. Effects on microbial population must be determined by daily total counts of viable organisms in sludge. For acceptable procedures for this test, see appendix item 8.

§ 163.62-9 Data requirements: Mobility.

(a) *General.* Pesticide residue movement may cause contamination of the food web (including nontarget crops), loss of usable land and water resources to man, and habitat loss to wildlife. Mobility studies include leaching, volatility, adsorption/desorption, and water dispersal of pesticides.

(b) *Leaching.* Leaching data are required to support registration of all formulated products intended for terrestrial noncrop, tree fruit/nut crop, and field/vegetable crop uses, and terrestrial/aquatic (forest) uses. Leaching through soil is dependent upon pesticide formulation, physical-chemical properties of the pesticide, and soil and environmental conditions. The pesticide shall be added to soil(s) corresponding to the highest recommend-

ed rate for a single application, and a laboratory leaching study shall be undertaken using radioisotopic or non-radioisotopic analytical techniques to provide a quantitative estimate of mobility in soil. Each study shall include soils as sand (agricultural), sandy loam, silt loam, clay, or clay loam having a pH of 4 to 8 with at least one soil having an organic matter content less than one percent. A minimum of four soils shall be used to study pesticide leaching, and each in a column of 30 cm height should be immediately eluted with 20 acre-inches (20.6 hectare-centimeters) of water. One of the above soils shall be used to study leaching of pesticide residues after the pesticide has been aged in soil under aerobic conditions (see paragraph (b) of § 163.62-8 for a description of soil temperature and moisture parameters) for 30 days prior to eluting with the equivalent of one-half acre-inch (0.52 ha-cm) water per day for 45 days. The preferred basic techniques for measuring leaching employ soil columns or soil thin-layer chromatography (soil TLC). Under certain circumstances, the soil column may be preferred for the study involving pesticide aging in soil. For acceptable procedures of this test, see appendix item 9.

(c) *Volatility.* Laboratory volatility studies using non-radioisotopic analytical techniques are required to support the registration of all formulated products intended for greenhouse use. Volatility field studies under any use conditions may be required for reentry assessment. Pesticides may be lost from treated areas by volatilization. Vapor pressure and adsorption characteristics of pesticides are important factors controlling volatilization. Other factors include soil texture, temperature, climate, and certain physical and chemical properties of pesticides. Air samples shall be monitored for residues at treated sites at the following time intervals: 0, 12 hours, 1 day, 2, 5, 7, 14, 21, and 30 days. For supplemental information for this test, see appendix item 10.

(d) *Adsorption/desorption.* A laboratory study using radioisotopic or non-radioisotopic analytical techniques is required to support the registration of all formulated products intended for all terrestrial uses, terrestrial/aquatic (forest) uses, aquatic uses, and aquatic impact uses (if the pesticides are discharge directly into the aquatic environment). Adsorption of pesticides to soil, sediment, and particulate matter influences the availability and concentration of pesticides in water as well as leaching and volatility of pesticides. Adsorption coefficients shall be determined using one soil (sediment) and at least four concentrations of pesticide in distilled water. The sediment selected for this study must be representative of the proposed use area (terrestrial/aquatic (forest), aquatic), and should be the same soil as used for the leaching study (paragraph (b) of this section). Additionally, sediments will be required if the sediment characteristics vary widely among proposed use sites. Desorption coefficients are to be determined on the same sediment. For acceptable procedures and supplemental information on this test, see appendix item 11.

(e) *Water dispersal.* A field study tailored to one or more representative sites is required to support the registration of all formulated products intended for aquatic uses and aquatic impact uses (if the pesticides are discharged directly into aquatic sites). Pesticides dispersed throughout the aquatic environment. This study shall establish pesticide residue concentration in water at selected distances from sites of exposure. Analysis of pesticide residues in water using a chemical or bioassary procedure of comparable sensitivity is required. Analysis of residue buildup in selected indicator organisms using chemical methods is required. For supplemental information for this test, see appendix item 12.

§ 163.62-10 Data requirements: Field Dissipation.

(a) *General.* A field dissipation study under actual use conditions is required to support the registration of all formulated products intended for terrestrial (except greenhouse) uses, aquatic uses, and terrestrial/aquatic (forest) uses. (See table 2 of §163.62-3.) Field studies determine pesticide dissipation and mobility in the environment which can proceed at different rates than under laboratory conditions. Decline curves obtained under field conditions define the duration of residues which are used in assessment of potential hazards. The potential hazards are reentry into the treated area, residues in rotational crops, residues in the food web, and loss of land and water resources. Dissipation may decrease these hazards through degradation processes in the treated area or may increase potential hazards in nontreated areas via mobility of the pesticide from the site of application. For these studies sufficient residue data shall be collected until 90 percent loss of the pesticide occurs or until patterns of formation and decline of degradation products are established or to the maximum time specified in paragraphs (b)(1) through (6) and paragraphs (c)(1) and (2) of this section. Sampling times include pre-application, day of application, and immediately post-application for each single or multiple application. The collection of succeeding samples is dependent upon degradation and metabolism characteristics and reentry considerations. identification of residues comprising more than 10 percent of initial application or 0.01 ppm, whichever is greater, is needed for the applicant to construct decline curves of residues in foliage, litter, soil, and water. For supplemental information for these tests, see appendix item 13.

(b) *Terrestrial.* Terrestrial field dissipation tests are specified below. If multiple applications are anticipated, all use patterns must be reflected in the study.

(1) *Field and vegetable crop uses.* Soil samples shall be taken in increments to a depth of 30 cm from sites in four agricultural use areas for a maximum test duration of 18 months.

(2) *Tree fruit and nut crop uses.* Soil samples shall be taken in increments to a depth of 30 cm from sites in two agricultural areas for a maximum test duration of 12 months.

(3) *Pasture land uses.* Soil samples shall be taken in increments to a depth of 30 cm from sites in three agricultural use areas for a maximum test duration of 12 months.

(4) *Domestic outdoor, parks, ornamental and turf uses.* Soil samples shall be taken in increments to a depth of 15 cm from sites in two use areas for a maximum test duration of 4 months.

(5) *Rights-of-way, shelterbelts and related uses.* Soil samples shall be taken in increments to a depth of 15 cm from sites in two use areas for a maximum test duration of 2 months.

(c) *Aquatic.* Aquatic field dissipation tests are designed to determine rate, mobility, and accumulation under actual field conditions as follows:

(1) *Aquatic food crop uses.* Soil samples shall be taken in increments to a depth of 15 cm from each of two agricultural use areas for a maximum test duration of 12 months. A minimum of four water samples shall be taken in duplicate for a maximum test duration of 1 month. The surface layer of water should not be included in the sample. For additional related data requirements, see §163.62-9(c) and §163.62-11(e). For supplementary information for these tests, see appendix item 14.

(2) *Aquatic noncrop uses.* A minimum of four water samples shall be taken, including two downstream, in duplicate, from two areas of intended use for a maximum test duration of 1 month. At each water sampling, fish (bottom, middle, and surface feeders, if available) shall be analyzed for whole body and edible tissue residues. The sediments shall be sampled at pretreatment and post-treatment at 7, 14, and 28 days, with sampling continued until a decline curve is established or for a maximum test duration of 6 months. When multiple applications are proposed, the duration of sediment sampling is to be extended by at least 3 months. In addition to the above requirements for aquatic noncrop uses, a soil dissipation study employing the

same sampling protocol as described for sediments in this section is required for products intended for application to ditchbanks and shorelines and for other unintentional direct aquatic applications. For supplemental information on this test, see appendix item 15.

(3) *Specialized aquatic uses.* For antifouling paints on ships and related protective or preservative uses, a laboratory study employing nonradioisotopic analytical techniques is required for the determination of movement of residues from treated surfaces in water. When any movement is detected, the data requirements for aquatic noncrop uses apply. (See paragraph (c)(2) of this section.)

(d) *Terrestrial/aquatic (forest).* Terrestrial/aquatic environments such as forests and reforestation sites require a special ecosystem residue study using the formulated pesticide product to assess environmental fate and hazard. Environmental components of the ecosystem residue study to be monitored include: foliage, leaf litter, litter-covered soil, exposed soil, standing (pond) water, moving (stream) water, sediments, fish, and other aquatic organisms. (For fish and other aquatic organism field studies, see *Accumulation. Special study.* §163.62-11(e).) One field location representative of the treated area is sufficient for the ecosystem residue study. Additional sites may be needed if the proposed use includes widely differing forest types. Data between various forest types or climates should be extrapolated. Sampling times include pre-application, date of application, and post-application, to construct decline curves for residues in foliage leaf litter, soil, and standing water. Three samples shall be collected during the first week post-application for the purpose of reentry assessment. When analyses of successive leaf litter, soil, and standing water samples show residues at the level of method sensitivity, and foliage samples indicate that greater than 90 percent of initial residues have dissipated, sampling may be terminated. Sampling need not be as frequent for sediments, running water, and aquatic organisms. To determine pesticide movement in forest floor environments, exposed soil shall be sampled to 15 cm, and litter-covered soil to 8 cm, over a 2-week period following precipitation. For an acceptable procedure for the study, see appendix item 16.

(e) *Aquatic impact uses.* Tests designed to determine dissipation rate, mobility, and accumulation of residues discharged or dispersed into the aquatic environment are enumerated in table 2 of §163.62-3(a)(1) and further explained in §163.62-6(c). In addition, the following are considered as:

(1) Direct discharge: pesticides passing through wastwater treatment systems and subsequently discharged into aquatic environments. The data requirements for direct discharge uses (§163.62-6(c)(2)) apply.

(2) Indirect discharge: pesticides disposed through discharges into wastewater treatment systems. The data requirements for indirect discharge uses (§163.62-6(c)(1)) apply.

(3) Wastewater treatment: pesticides used as treatments in wastewater treatment systems. The data requirements for wastewater treatment uses (§163.62-6(c)(1)) apply.

(f) *Combination and tank mixes.* A laboratory or field soil dissipation study, using the formulated product (preferred) or the non-radiolabeled technical grade of each active ingredient, is required to support the registration of all formulated products intended for combination and tank mixtures in addition to any other required environmental chemistry studies. This study shall compare the dissipation characteristics of each active ingredient of the mixture when applied to the soil as a mixture with the separate dissipation characteristics of each active ingredient in soil when applied individually. The active ingredients shall be applied both individually and as a mixture at recommended field application rates to light and heavy-textured soils. If a field study is selected, soil should be sampled to a depth of 15 cm. If a laboratory study is selected, soil should be sampled to the bottom of the container. For either study, sampling should be continued until a residue decline curve is established or for a maximum test duration of 6 months. For supplemental information for these tests, see appendix item 17.

(g) *Long-term study.* If pesticide residues do not reach fifty percent dissipation in soil prior to recommended subsequent application on the sites utilized for the field dissipation study described in paragraphs (b)(1) or (c)(1) of this section, then a long-term field dissipation study on these same treated sites is required under actual use conditions from multiple or yearly applications to field and vegetable crops and aquatic food crops. The total duration of this study is not required to exceed 3 years or extend 2 years beyond the field dissipation study described in paragraphs (b)(1) or (c)(1) of this section. For supplemental information on this study, see appendix item 18.

§ 163.62-11 Data requirements: Accumulation.

(a) *General.* Pesticides introduced in the environment may enter and accumulate in food webs. Studies specified under paragraphs (b), (c), (d), and (e) of this section are needed to determine this accumulation, and to assess potential adverse effects on nontarget organisms indicated by rotational crops, irrigated crops, and fish.

(b) *Rotational crops.* The studies described in this paragraph are required to support the registration of all formulated products intended for field/vegetable and aquatic food crop uses. These studies will determine if pesticide residue uptake occurs in rotational crops and emergency replantings. The applicant must identify crops that can be rotated in the proposed use areas. A laboratory or greenhouse study that utilizes a sandy loam soil treated with radiolabeled pesticide at a rate equivalent to that expected under actual use conditions is required. Following treatment, the pesticide shall be aged under aerobic conditions for a time approximating the anticipated cultural practice; for example, 1 year for corps rotated the following year, 120 days for crops rotated immediately after harvest, and 30 days for assessing circumstances of crop failure. Representative root, small grain, and leafy vegetable crops at the above times shall be planted and periodically analyzed to maturity. When residues are found in the crops analyzed in the laboratory or greenhouse study, then a field study using formulated products shall be undertaken to determine if residues would occur in subsequent crops under actual use conditions. An additional crop residue study under actual field use conditions is required for those practices where a subsequent crop is treated with the same active ingredient as the initial crop. This study is not required for a cover crop if topically plowed under and not grazed. For supplemental information for these studies, see appendix item 19.

(c) *Irrigated crops.* A crop residue study under actual field use conditions is required to support the registration of all formulated products intended for aquatic food or aquatic noncrop uses, or for use in holding ponds or effluent and other discharged sources used to irrigate crops. Representative crops shall be selected for this study in which residue uptake and residue levels are determined in those selected crops receiving water via artificial means or irrigation from treated areas or sources.

(d) *Fish accumulation.* Accumulation of residues in nontarget aquatic organisms is an indication of contamination of the food web. This laboratory study employing radioisotopic or nonradioisotopic analytical techniques is required to support the registration of all formulated products intended for terrestrial noncrop, tree fruit/nut crop, and field/vegetable crop uses; aquatic food crop and noncrop uses; terrestrial/aquatic (forest) uses; and aquatic impact (direct discharge) uses. Two exposure systems are required: flow-through (with constant concen-

tration of pesticide in aqueous solution) and static (with ambient concentration of residues from treated soil. Sunfish are the preferred species in the flow-through system, and catfish are the required species in the static system. For the static system, sandy loam soil shall be treated at the use rate, and aged under aerobic conditions for 2 to 4 weeks prior to the initiation of fish exposure. For data requirements of an aquatic food crop use, treated soil shall be flooded with water for 30 days after aerobic aging prior to initiation of fish exposure. Exposure duration is 30 days with suggested sampling times for fish, water, and soil of 0, 1, 3, 7, 10, 14, 22, and 30 days. Fish and water samples shall be taken on 0, 1, 3, 7, 10, and 14 of residue depuration (dissipation). Soil and water samples shall be obtained prior to fish exposure interval. The amount and identity of the residue in water, soil, whole body fish, edible tissue, and viscera at each sample interval shall be determined. (See § 163.72-6, Hazard Evaluation Subpart E, for additional bioaccumulation requirements.) For acceptable procedures and supplemental information on this study, see appendix item 20.

(e) *Special studies—aquatic noncrop uses.* Pesticides employed for aquatic noncrop uses require a laboratory fish accumulation study (see paragraph (d) of this section) and a field study, under actual use conditions, employing nonradioisotopic analytical techniques. For the latter study, samples of water and fish (including a bottom, middle, and surface-feeding fish) shall be analyzed for amount and identity of residues. (See § 163.72-5, Hazard Evaluation Subpart E, for additional data requirements on pesticide environmental distribution potential, persistence, and bioaccumulation as needed to evaluate hazards to fish and wildlife).

§ 163.62-12 Data requirements: Reentry [Reserved]

§ 163.62-13 Data requirements: Disposal and storage.

Environmental chemistry data to support label statements on disposal and storage are required to support the registration of all manufacturing-use products and all formulated products. Specific data requirements and testing protocols needed to generate these data are being developed. In addition, however, applicants are advised that the Resources Conservation and Recovery Act of 1976 (Pub. L. 94-580) provides for the development of standards for those who generate, transport, treat, store, or dispose of hazardous wastes. Disposal instructions on the labels of pesticide containers will need to be consistent with such standards.

APPENDIX TO ENVIRONMENTAL CHEMISTRY

General. The wide range of chemical characteristics of pesticides and their diversity of use preclude defining universally acceptable protocols which will satisfy environmental chemistry data requirements set forth in §§ 163.62-2 through 163.62-13. The following information, consisting of a series of items keyed by subject heading to the appropriate paragraph of the environmental chemistry guidelines, provides referenced examples of published literature containing acceptable procedures and supplemental background or ancillary material which may be consulted in developing acceptable protocols. The Agency recognizes that more than one referenced study may be utilized in developing an acceptable protocol in a given area. In all cases, if the referenced procedure(s) utilized to develop an acceptable protocol contains other information which is inconsistent with test standards and requirements as specified in the guidelines, this information must be modified accordingly to bring it into conformity with the intent and substance of the test requirements in the guidelines.

A detailed discussion of many of the tests described in §§ 163.62-1 through 163.62-11 is provided in the appendix to the previously proposed registration guidelines: 40 FR 26802, June 25, 1975, pages 26878-26896.

Item No. 1 *Hydrolysis.* The following reference contains an acceptable procedure for conducting hydrolysis studies:

Krzeminski, S. F., C. K. Brackett, and J. D. Fisher. 1975. Fate of microbial 3-isothiazolone compounds in the environment: modes and rates of dissipation. *J. Agr. Food Chem.* 23:1060-1068.

Supplemental information on theory and development of rate constants is given in the following reference:

Gomaa, H. M., I. H. Suffet, and S. D. Faust. 1969. Kinetics of hydrolysis of diazinon and diazoxon. *Residue Rev.* 29:171-190.

Item 2. *Photolysis.* The following references contain acceptable procedures for conducting photolysis studies:

Koshy, K. T., A. R. Friedman, A. L. van der silk, and D. R. Craber. 1975. Photolysis of benzoic acid 2-(2,4,6-trichlorophenyl)-hydrazide. *J. Agr. Food Chem.* 23:1084-1088.

Nakagawa, M., and D. G. Crosby. 1974. Photodecomposition of nitrofen. *J. Agr. Food Chem.* 22:849-853.

Niles, G. P., and M. J. Zabik. 1975. Photochemistry of bioactive compounds. Multiphase photodegradation and mass spectral analysis of basagran. . J. Agr. Food Chem. 23:410-415.

Newsom, H. C., and W. G. Woods. 1973. Photolysis of the herbicide dinitramine (N^3, N^3-diethyl - 2,4 - dinitro - 6 - trifluoromethyl - m - phenylenediamine). *J. Agr. Food Chem.* 21:598-601.

Su, G. C. C., and M. J. Zabik. 1972. Photochemistry of bioactive compounds. Photolysis of arylamidine deviative in water. *J. Agr. Food Chem.* 20:320-323.

Supplemental information for developing a protocol to conduct a photolysis study for reentry assessment is given in the following references:

Crosby, D. G., and K. W. Moilanen. 1974. Vapor-phase photodecomposition of aldrin and dieldrin. *Arch. Environ. Contam. Toxicol.* 2:62-74.

Soderquist, C. J., D. G. Crosby, K. W. Moilanen, J. N. Seiber, AND J. E. Woodrow. 1975. Occurrence of trifluralin and its photoproducts in air. *J. Agr. Food Chem.* 23:304-309.

Item No. 3 *Soil metabolism.* The following references contain acceptable procedures for conducting soil metabolism studies:

Bartha, R., and D. Pramer. 1965. Features of flask and method for measuring the persistence and biological effects of pesticides in soil. *Soil Sci.* 100:68-70.

Betts, P. M., C. W. Griddings, and J. R. Fleeker. 1976. Degradation of 4-aminopyridine in soil. *J. Agr. Food Chem.* 24:571-574.

Fleeker, J. R., H. M. Lacy, I. R. Schultz, and E. C. Houkom. 1974. Persistence and metabolism of thiophanate-methyl in soil. *J. Agr. Food Chem.* 22:592-595.

Gingerich, L. L., and R. L. Zimdahl. 1976. Soil persistence of isopropalin and oryzalin. *J. Agr. Food Chem.* 24:431-434.

Gowda, J. K. S., and N. Sethunathan. 1976. Persistence of endrin in Indian rice soils under flooded conditions. *J. Agr. Food Chem.* 24:750-753.

Messersmith, C. G., O. C. Burnside, and T. L. Lavy. 1971. Biological and nonbiological dissipation of trifluralin from soil. *Weed Sci.* 19:285-290.

Parr, F. F., and S. Smith. 1969. A multipurpose manifold assembly: Use in evaluating microbiological effects of pesticides. *Soil Sci.* 107:271-276.

Parr, J. F., and S. Smith. 1973. Degradation of trifluralin under laboratory conditions and soil anaerobiosis. *Soil Sci.* 115:55-63.

Schooley, D. A., K. M. Creswell, L. E. Starger, and G. B. Quislad. 1975. Environmental degradation of the insect growth regulator isopropyl (2-*E*, 4-*E*)-11-methoxy-3,7,11 - trimethyl-2,4-dodecadienoate (methoprene). IV. Soil metabolism. *J. Agr. Food chem.* 23:369-373.

Smith, R. A., W. S. Belles, K. W. Shen, and W. G. Woods. 1973. The degradation of dinitramine (N^4 N^3-diethyl 2,4-dinitro-6-trifluoromethyl-m-phenylenediamine) in soil. *Pest. Biochem. Physiol.* 3:278-288.

Kazano, H., P. C. Kearney, and D. D. Kaufman. 1972. Metabolism of methylcarbamate insecticides in soils. *J. Agr. Food chem.* 20:975-979.

Stevenson, P. J. 1965. Gross chemical fractionation of organic matter. Pp. 1409-1421 *in* C. A. Black, D. D. Evans, J. L. White, L. E. Ensminger, and F. E. Clark (eds). Methods of Soil Analysis. Amer. Soc. Agron. Publ. No. 9. Madison, Wis.

Schooley, D. A., K. M. Creswell, L. E. Slaiger, and G. B. Quistad. 1975. Environmental degradation of the insect growth regulator isopropyl (2-*E*, 4-*E*)-11-methoxy-3,7,11-trimethyl-2,4-dococadienoate (methoprene). IV. Soil Metabolism. *J. Agr. Food Chem.* 23:369-373.

Item No. 4. *Anaerobic aquatic metabolism.* The following references contain acceptable procedures for conducting anaerobic aquatic metabolism studies:

Gowda, T. K. S, and N. Sethunathan. 1976. Persistence of endrin in Indian rice soils under flooded conditions. *J. Agr. Food Chem.* 24:750-753.

Hance, R. J., and G. Chesters. 1969. The fate of hydroxyatrazine in a soil and a lake sediment. *Soil Biol. Biochem.* 1:309-315.

Miyazaki, S., H. C. Sikka, and R. S. Lynch. 1975. Metabolism of dichlobenil by microorganisms in the aquatic environment. *J. Agr. Food Chem.* 23:365-368.

Sikka, H. C., and C. P. Rico. 1973. Persistence of endothall in aquatic environment as determined by gas-liquid chromatography. *J. Agr. Food Chem.* 21:842-846.

Item No. 5. *Aerobic aquatic metabolism.* The following references contain acceptable procedures for conducting aerobic aquatic metabolism studies:

Krzeminski, S. F., C. K. Brackett, and J. D. Fisher. 1975. Fate of microbial 3-isothiazolone compounds in the environment: modes and rates of dissipation. *J. Agr. Food Chem.* 23:1060-1068.

Schaffer, C. H., and E. F. Dupras, Jr. 1976. Factors affecting the stability of dimilin in water and the persistence of dimilin in field waters. *J. Agr. Food Chem.* 24:733-739.

Item No. 6. *Effects of microbes on pesticides.* The followng references contain acceptable procedures for isolating microorganisms from the soil for pure or mixed culture studies:

Curl, E. A., and R. Rodriguez-Kabana. 1977. Herbicide—Plant disease relationships. Pp. 172-191 *in* B. Truelove (ed). Research Methods in Weed Science. *S. Weed Sci. Soc.* Second edition. Auburn Printing, Inc.: Auburn, Ala.

Goswami, K. P., and R. E. Green. 1971. A simple automatic soil percolator. *Soil Biol. Biochem.* 3:389-391.

Kaufman, D. D. 1966. An inexpensive positive pressure, soil perfusion system. *Weeds.* 14:90-91.

Sharp, R. F., and B. P. Taylor. 1969. A new soil perculator for the elective culture of soil organisms. *Soil Biol. Biochem.* 1:191-194.

The following references contain acceptable procedures for determining the effects of pure or mixed microbial cultures on pesticides:

Benezet, H. J., and F. Matsumura. 1974. Factors influencing the metabolism of mexacarbate by microorganisms. *J. Agr. Food Chem.* 22:427-430.

Furukawa, K., and F. Matsumura. 1976. Microbial metabolism of polychlorinated biphenyls. Studies on the relative degradability of polychlorinated biphenyl components by *Alkaligenes.* sp. *J. Agr. Food Chem.* 24:251-256.

Sikka, H. C., and J. Saxena. 1973. Metabolism of endothall by aquatic organisms. *J. Agr. Food Chem.* 21:402-406.

Van Alfen, N. K., and T. Kosuge. 1974. Microbial metabolism of the funcigide 2,6-dichloro-4-nitroaniline. *J. Agr. Food Chem.* 22:221-224.

The following reference gives an acceptable example for submitting descriptive characteristics of isolates in lieu of generic classification:

Bollag, J., E. J. Czaplicki, and R. D. Minard. 1975. Bacterial metabolism of 1-naphthol. *J. Agr. Food Chem.* 23:85-90.

Item No. 7. *Effects of pesticides on microbes.* The following references contain supplemental information for developing acceptable protocols to determine the effects of pesticides on microbial functions:

Anderson, J. R. 1973. A system for evaluating effects of pesticides on soil microbial activity. *Bull. Ecol. Res. Comm.*. 17:470-472. (Now: Ecological Bulletins, Swedish Natural Research Council.)

Balandreau, J., and Y. Dommergues. 1973. Assaying nitrogenase (C_2H_2) activity in the field. *Bull. Ecol. Res. Comm.*. 17:247-254. (Now: Ecolog. Bull., Swed. Natural Res. Council.)

Bohool, B. B., and E. L. Schmidt. 1973. A fluorescent antibody technique determination of growth rates of bacteria in soil. *Bull. Ecol. Res. Comm.* 17:336-338. (Now: Ecolog. Bull., Swed. Natural Res. Council.)

Casida, L. E., Jr. 1968. Methods for the isolation and estimation of activity of soil bacteria. Pp. 97-122 *in* Gray, T.R.G. and P. Parkinsen (eds), The Ecology of Soil Bacteria. University of Toronto Press: Canada.

Curl, E. A., and R. Rodriguez-Kabana. 1971. Microbial Interactions. Pp. 162-194 *in* R. E. Wilkinson (ed), Research methods in Weed Science. *S. Weed Sci. Soc.* PCP Enterprises, Inc: Atlanta, Ga.

Gray, T. R. G., P. Baxby, I. R. Hill, and M. Goodfellow. 1969. Direct observation of bacteria in soil *In.* The Ecology of Soil Bacteria. Liverpool Press: Canada.

Jensen, V. 1969. The plate count technique. *In.* The Ecology of Soil Bacteria. Liverpool Press: Canada.

Knowles, R., R. Brouzes, and P. O'Toole. 1973. Kinetics of nitrogen fixation and acetylene reduction, and effects of oxygen and of acetylene on these processes, in a soil system. *Bull. Ecol. Res. Comm.* 17:255-262 (Now: Ecolog. Bull., Swed. Natural Res. Council.

Matulewich, V. A., P. F. Strom, and M. S. Finstein. 1975. Length of incubation for enumerating nitrifying bacteria in various environments. *Appl. Microbiol.* 29:265-268.

Pochon, J. and P. Tardieux. 1962. Enrichment techniques and the elective cultivation of bacteria. Pp. 123-137 *in.* Gray, T. R. G. and D. Parkinsen (eds), The Ecology of Soil Bacteria. University of Toronto Press: Canada.

Item No. 8. *Activated sludge metabolism.* The following references contain acceptable procedures for conducting activated sludge studies:

Krzeminski, S. F., C. K. Brackett, and J. D. Fisher. 1975. Fate of microbial 3-isothiazolone compounds in the environment: modes and rates of dissipation. *J. Agr. Food Chem.* 23:1060-1068.

Soap and Detergent Association, Test Procedure and Standards—ABS and LAS Biodegradability. Scientific and Technical Report No. 3. January, 1966.

The following reference contains an acceptable procedure for determining plate counts of organisms in activated sludge studies:

American Public Health Association. 1965. Standard Methods for the Examination of Water and Wastewater. P. 592 *in.* Twelfth Edition.

Item No. 9. *Leaching.* The following references contain acceptable procedures for conducting mobility (leaching) studies:

Helling, C. S. 1971. Pesticide mobility in soils I. Parameters of soil thin-layer chromatography. *Soil Sci. Soc. Amer. Proce.* 35:732-737.

Krzeminski, S. F., C. K. Brackett, and J. D. Fisher. 1975. Fate of microbial 3-isothiazolone compounds in the environment: modes and rates of dissipation. *J. Agr. Food Chem.* 23:1060-1068.

Lichtenstein, E. P., K. R. Schulz, and T. W. Fuhremann. 1972. Movement and fate of dyfonate in soils under leaching and non-leaching conditions. *J. Agr. Food Chem.* 20:831-838.

The following reference also contains an acceptable procedure for conducting this study. The use of bioassay techniques mentioned is not acceptable as a procedure.

Weber, J. B. and T. F. Peeper. 1977. Herbicide mobility in soils. Pp. 73-78 *in* B. Truelove (ed), Research Methods in Weed Science. *S. Weed Sci. Soc.* Second edition. Auburn Printing, Inc.: Auburn, Ala.

Item No. 10. *Volatility.* Acceptable procedures for mobility-volatility studies are not available. The following references contain supplemental information for developing a protocol to conduct field volatility studies:

Harper, L. A., A. W. White, Jr., R. R. Bruce, A. W. Thomas, and R. A. Leonard. 1976. Soil and microclimate effects on trifluralin volatilization. *J. Environ. Qual.* 5:236-242.

Parmele, L. H., E. R. Lemon, and A. W. Taylor. 1972. Micrometeorological measurement of pesticide vapor flux from bare soil and corn under field conditions. *Water, Air, and Soil Pollut.* 1:433-451.

Soderquist, C. J., D. G. Crosby, K. W. Moilanen, J. N. Seiber, and J. E. Woodrow. 1975. Occurrence of trifluralin and its photo-products in air. *J. Agr. Food Chem.* 23:304-309.

The following references contain supplemental information for determining pesticide volatility in the laboratory as a preliminary study:

Kearney, P. C. and A. Konston. 1976. A simple system to simultaneously measure volatilization and metabolism of pesticides from soils. *J. Agr. Food Chem.* 24:424-426.

Spencer, W. F. and M. M. Claith. 1974. Factors affecting vapor loss of trifluralin from soil. *J. Agr. Food Chem.* 22:987-991.

Item No. 11. *Adsorption/Desorption.* The following references contain acceptable procedures for conducting adsorption/desorption studies:

Aharonson, N. and U. Kafkafi. 1975. Adsorption, mobility and persistence of thiabendazole and methyl 2-benzimidazole carbamate in soils. *J. Agr. Food Chem.* 23:720-724.

Harvey, R. G. 1974. Soil adsorption and volatility of dinitroaniline herbicides. *Weed Sci.* 22:120-124.

Murray, D. S., P. W. Santelmann, and J. M. Davidson. 1975. Comparative adsorption, desorption, and mobility of dipropetryn and prometryn in soil. *J. Agr. Food Chem.* 23:578-582.

Saltzman, S., L. Kliger, and B. Yaron. 1972. Adsorption, desorption of parathion as affected by soil organic matter. *J. Agr. Food Chem.* 20:1224-1226.

Wu, C. H., N. Buehring, J. M. Davidson, and P. W. Santelmann. 1975. Napropamide adsorption, desorption, and movement in soils. *Weed Sci.* 23:454-457.

The following references contain supplemental information for developing a protocol for adsorption/desorption studies:

Bailey, G. W. and J. L. White. 1970. Factors influencing the adsorption and movement of pesticides in soils. *Residue Rev.* 32:29-92.

Weber, J. B. 1977. Soil properties, herbicide sorption, and model soil systems. Pp. 59-72 *in* B. Truelove (ed). Research Methods in Weed Science. *S. Weed Sci. Soc.* Second edition. Auburn Printing, Inc.: Auburn, Ala.

Item No. 12. *water dispersal.* Acceptable procedures for this study are not available. The following references contain supplemental information for developing a protocol for conducting water dispersal studies:

Comes, R. D., V. F. Burns, and A. D. Kelly. 1976. Residues and persistence of glyphosate in irrigation water. *Weed Sci.* 18:47-50.

Demint, R. J., A. Frank, and R. D. Comes. 1970. Amitrole residues and rate of dissipation in irrigation water. *Weed Sci.* 18:439-442.

Frank, P. A., R. J. Demint, and R. D. Comes. 1970. Herbicides in irrigation water following canal-bank treatment for weed control. *Weed Sci.* 18:687-692.

Schaefer, C. H. and E. F. Dupras, Jr. 1976. Factors affecting the stability of dimilin in water and the persistence of dimilin in field waters. *J. Agr. Food Chem.* 24:733-739.

Item No. 13. *Field dissipation.* Acceptable procedure for field dissipation studies are not available. The following references contain supplemental information for developing a protocol for conducting such studies:

Caro, J. H., H. P. Freeman, and B. C. Turner. 1974. Persistence in soil and losses in runoff of soil-incorporated carbaryl in a small watershed. *J. Agr. Food Chem.* 22:860-863.

Miller, J. H., T. J. Monaco, and T. J. Sheets. 1976. Studies on nitralin residues in soils. *Weed Sci.* 24:288-291.

Polzin, W. J., I. F. Brown, Jr., J. A. Manthey, and G. W. Probst. 1971. soil persistence of fungicides—experimental design, sampling, chemical analysis and statistical evaluation. *Pest. Monit. J.* 4:209-215.

Item No.4. *Aquatic food crop.* Acceptable procedures for aquatic crop studies are not available. the following references contain supplemental information for developing a protocol for conducting such studies:

Demint, R. J., J. C. Pringle, Jr., A. Hattrup, V. F. Burns, and P. A. Frank. 1975. Residues in crops irrigated with water containing trichloroacetic acid. *J. Agr. Food Chem.* 23:81-84.

Rice, C. P., H. C. Sikka, and R. S. Lynch. 1974. Persistence of dichlobenil in a farm pond. *J. Agr. Food Chem.* 22:533-534.

Schaefer, C. H., and E. F. Dupras, Jr. 1976. Factors affecting the stability of dimilin in water and the persistence of dimilin in field waters. *J. Agr. Food Chem.* 24:733-739.

Sikka, H. C., and C. P. Rice. 1973. Persistence of endothall in aquatic environment as determined by gas-liquid chromatography. *J. Agr. Food Chem.* 21:842-846.

Item No. 15 *Aquatic noncrop.* Acceptable procedures for aquatic noncrop studies are not available. The following references contain supplemental information for developing a protocol for conducting such studies:

Demint, R. J., J. C. Pringle, Jr., A. Hattrup, V. F. Burns, and P. A. Frank. 1975. Residues in crops irrigated with water containing trichloroacetic acid. *J. Agr. Food Chem.* 23:81-84.

Rice, C. P., H. C. Sikka, and R. S. Lynch. 1974. Persistence of dichlobenil in a farm pond. *J. Agr. Food Chem.* 22:533-534.

Schaefer, C. H., and E. F. Dupras, Jr. 1976. Factors affecting the stability of dimilin in water and the persistence of dimilin in field waters. *J. Agr. Food Chem.* 24:733-739.

Sikka, H. C., and C. P. Rice. 1973. Persistence of endothall in aquatic environment as determined by gas-liquid chromatography. *J. Agr. Food Chem.* 21:842-846.

Item No. 16 *Terrestrial/aquatic (forest) dissipation.* An acceptable procedure for conducting terrestrial/aquatic (forest) dissipation studies can be found in the following reference:

Giles, P. H., Jr. 1970. The ecology of a small forested watershed treated with the insecticide malethion-S 35. Wildlife Monographs No. 24. The Wildlife Society: Washington, D.C.

Information contained in the reference is more extensive than generally required. However, the basic design is acceptable. The applicant is not required to use radiolabeled compounds as were used in this study.

Item No. 17 *Combination and tank mixes.* Acceptable procedures to conduct a combination or tank mix study are not available. The following references contain supplemental information for developing a protocol for conducting these studies:

Bartha, R., and D. Pramer. 1965. Features of a flask method for measuring the persistence and biological effects of pesticides in soil. *Soil Sci.* 100:68-70.

Kaufman, D. D., J. Blake, and D. E. Miller. 1971. Methyl carbamates affect acylanilide herbicide residues in soil. *J. Agr. Food Chem.* 18:204-206.

Kaufman, D. D., P. C. Kearney, D. W. VonEndt, and D. E. Miller. 1970. Methylcarbamate inhibition of phenylcarbamate metabolism in soil. *J. Agr. Food Chem.* 18:513-519.

Polzin, W. J., I. F. Brown, Jr., J. A. Manthey, and G. W. Probst. 1971. Soil persistence of fungicides—experimental design, sampling, chemical analysis and statistical evaluation. *Pest. Monit. J.* 4:209-215.

Item No. 18 *Long term study.* Acceptable procedures to conduct a long term study are not available. The following references contain supplemental information for developing a protocol to conduct this study:

Burnside, O. C. 1974. Trifluralin dissipation in soil following repeated annual applications. *Weed Sci.* 22:374-377.

Caro, J. H., H. P. Freeman, and B. C. Turner. 1974. Persistence in soil and loss in runoff of soil-incorporated carbaryl in a small watershed. *J. Agr. Food Chem.* 22:860-863.

Demint, R. J., J. C. Pringle, Jr., A. Hattrup, V. F. Burns, and P. A. Frank. 1975. Residues in crops irrigated with water containing trichloroacetic acid. *J. Agr. Food Chem.* 23:81-84.

Miller, C. H., T. J. Monaco, and T. J. Sheets. 1976. Studies on nitralin residues in soils. *Weed Sci.* 24:286-291.

Polzin, W. J., I. F. Brown, Jr., J. A. Manthey, and G. W. Probst. 1971. Soil persistence of fungicides—experimental design, sampling, chemical analysis and statistical evaluation. *Pest. Monit. J.* 4:209-215.

Rice, C. P., H. C. Sikka, and R. S. Lynch. 1974. Persistence of dichlobenil in a farm pond. *J. Agr. Food Chem.* 22:533-534.

Schaefer, C. H., and E. F. Dupras, Jr. 1976. Factors affecting the stability of dimilin in water and the persistence of dimilin in field waters. *J. Agr. Food Chem.* 24:733-739.

Sikka, H. C. and C. P. Rice. 1973. Persistence of endothall in aquatic environment as determined by gas-liquid chromatography, *J. Agr. Food Chem.* 21:842-846.

Item No. 19. *Accumulation—rotational crops.* Acceptable procedures to study the accumulation of residues in rotational crops are not available. The following references contain supplemental information for developing a protocol for conducting such studies:

Burnside, O. C. 1974. Trifluralin dissipation in soil following repeated annual applications. *Weed Sci.* 22:374-377.

Burnside, O. C., C. R. Fenster, and G. A. Wicks. 1971. Soil persistence of repeated annual applications of atrazine. *Weed Sci.* 19:290-293.

Sirons, G. J., R. Frank, and T. Sawyer. 1973. Residues of atrazine, cyanazine, and their phytotoxic metabolites in clay loam soil. *J. Agr. Food Chem.* 21:1016-1020.

Item No. 20. *Accumulation—fish.* The following references contain acceptable procedures for conducting the sunfish study. Acceptable procedures for conducting the catfish study are not available.

Kennedy, G. L., M. L. Keplinger, O. E. Fancher, J. D. Stone, and J. C. Calandra. 1975. Metabolic studies with dinitramide. *Toxicol and Appl Pharm.* 33:341-349.

Krzeminski, S. F., C. K. Brackett, and J. D. Fisher. 1975. Fate of microbial 3-isothiazolone compounds in the environment: modes and rates of dissipation. *J. Agr. Food chem.* 23:1060-1068.

Mount, D. I., and W. D. Brungs. 1967. A simplified dosing apparatus for fish toxicology studies. *Water Res.* 1:21-29.

Supplemental information for developing a protocol for a fish accumulation study is given the following references:

Macek, K. J. 1975. Bioaccumulation of ^{14}C-pesticides by bluegill sunfish during continuous aqueous exposure. Pp. 119-142 *in* G. D. Veith and D. E. Konasewich (eds), Structure Activity Correlations in Studies of Toxicity and Bioconcentration with Aquatic Organisms. Great Lakes Advisory Board, International Joint Commission: Windsor, Ontario, Canada.

Schultz, D. P. 1973. Dynamics of a salt of (2,4-dichlorophenoxy)-acetic acid in fish, water and hydrosol. *J. Agr. Food Chem.* 21:186-192.

Item No. 21. *Reentry.* A presentation of the present state of the art, recent experimental activity, and suggested approaches to reentry assessment can be found in the following reference:

Federal Working Group on Pest Management. 1974. Report of the Task Group on Occupational Exposure to Pesticides. U.S. Environmental Protection Agency. Washington, D.C.

An acceptable procedure for establishing dislodgable residues is given in the following reference:

Gunther, F. A., W. E. Westlake, and J. H. Barkley. 1973. Establishing dislodgable pesticide residues on leaf surfaces. *Bull. Environ. Contam. Toxicol.* 9(4):234-249.

Also included in this reference are discussions of sampling procedures and a description of leaf punches used in sample collection. Although additional research may uncover other methods more predictive of the respiratory and dermal dose variable, at present these techniques appear to be most suitable.

SPECIAL CHEMISTRY REQUIREMENTS

§ 163.63-1 Tolerance clearance.

(a) A pesticide, intended for use on food or feed or whose use may reasonably be expected to result (directly or indirectly) in residues of the pesticide becoming a component of the food or feed, will not be registered unless a tolerance or an exemption from the requirement to obtain a tolerance has been issued by the Agency, as provided by sections 406, 408, or 409 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 346, 346a, and 348). A temporary tolerance may be established in conjunction with an experimental use permit. (See part 172 of this chapter).

(b) Any change in the use of the pesticide will require an evaluation on the part of the Agency to determine whether or not the residues for the new use will exceed the level of the previously-established tolerances. Residue data may accordingly be required to support the application for registration of a new use.

§ 163.64-1 Pesticides on tobacco.

(a) Use of a pesticide on tobacco does not require a tolerance or an exemption from the requirement to obtain a tolerance. Nonetheless, data are needed to assess the exposure of man to residual products upon the consumption of tobacco, including: A residue profile for the tobacco and (under certain conditions) its smoke, for those pesticide products intended for use on tobacco. In addition to residue determinations of the active ingredient, this residue profile must include, when detected, polar and nonpolar plant metabolities of the active ingredient, translocated degradation products from soil, and photodegradation products. Radiosotopic techniques will normally be required. If residues at 0.1 ppm (mg/kg) or more are detected, analytical methods similar to those available in the sources listed in the appendix must be developed to characterize the residual products.

(b) If the pesticide use pattern is a foliar treatment or application to soil, a study on follow-up tobacco is also required to determine whether detectable residues can be expected in the green leaf.

(c) Data from the following studies must be submitted to show conclusively the level of residue likely to result from the use of the product, including:

(1) Total residues on green freshly-harvested tobacco. If residues at 0.1 ppm (mg/kg) or more are detected, the determinations of paragraph (c)(2) of this section are required.

(2) Total residues on cured or dried tobacco. If residues at 0.1 ppm (mg/kg) or more are detected, the determinations in paragraph (c)(3) of this section are required.

(3) Total residues in smoke from cured or dried tobacco. If residues at 0.1 ppm (mg/kg) or more (dried tobacco basis) are detected in smoke, the determinations in paragraph (c)(4) of this section are required.

(4) Pyrolysis products derived from the active ingredient that occur at 0.1 ppm (mg/kg) or more (dried tobacco basis) must be identified, and subchronic inhalation studies utilizing the smoke be conducted. (See §163.82-4.)

APPENDIX TO SPECIAL CHEMISTRY REQUIREMENTS

The applicant is referred to the following published sources for analytical methods which are considered acceptable for determining residues on a variety of substrates and which may be adaptable for the determination of residues on tobacco.

Horowitz, W. 1975. Official Methods of Analysis. 12th ed., Association of Official Analytical Chemists, Post Office Box 540, Benjamin Franklin Station, Washington, D.C. 20044.

Food and Drug Administration. 1968-1975. Pesticide Analytical Manual. Food and Drug Administration, Rockville, Md. 20852.

Zweig, G., and J. Sherma. 1963-1976. Analytical Methods for Pesticides and Plant Growth Regulators. Continuing Series. Academic Press: New York, N.Y.

The applicant is referred to the following published source for additional information:

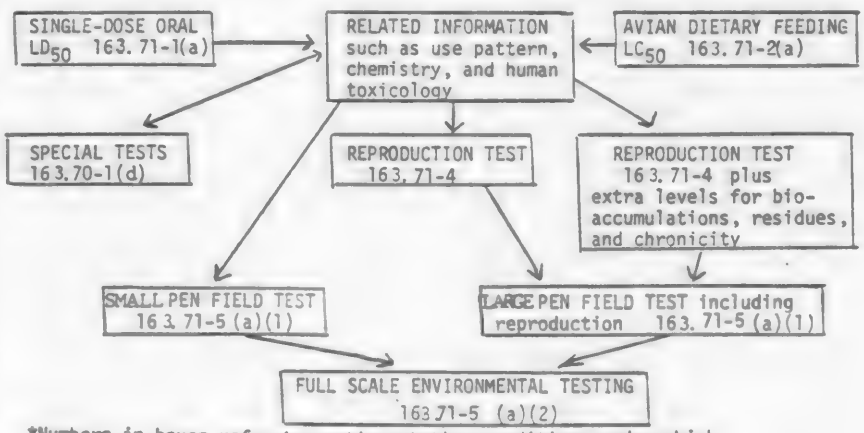
Guthrie, F. E., and T. G. Bowery. 1967. Pesticide residues in tobacco. Residue Rev. 19:31-56.

Subpart E—Hazard Evaluation: Wildlife and Aquatic Organisms

§ 163.70-1 General requirements.

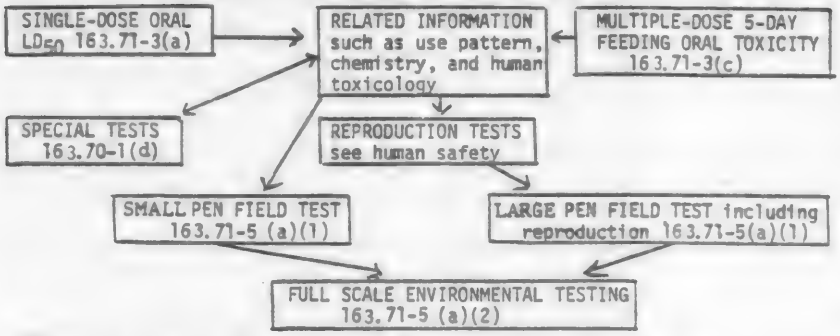
(a) *Scope.* §§163.71-1 through 163.71-5 and §§163.72-1 through 163.72-6 specify the data on toxicity to wildlife and aquatic organisms which are required to support to registration of a pesticide product. Each section specified the conditions under which the specific data are required. Figures 1, 2, and 3 illustrate the hierarchial testing scheme for avian wildlife, wild mammals, and aquatic organisms, respectively. The tests fall into a spectrum from the least expensive and progress (when needed) to the more costly. The sequence also progresses from the basic laboratory tests to the applied field tests. Based upon the conditions under which specific data are required, each sequential test will occur at a much lower frequency than the preceding test.

Figure 1 - AVIAN TESTING FLOW CHART*


SINGLE-DOSE ORAL LD50 163.71-1(a)
RELATED INFORMATION such as use pattern, chemistry, and human toxicology
AVIAN DIETARY FEEDING LC50 163.71-2(a)
SPECIAL TESTS 163.70-1(d)
REPRODUCTION TEST 163.71-4
REPRODUCTION TEST 163.71-4 plus extra levels for bio-accumulations, residues, and chronicity
SMALL PEN FIELD TEST 163.71-5(a)(1)
LARGE PEN FIELD TEST including reproduction 163.71-5(a)(1)
FULL SCALE ENVIRONMENTAL TESTING 163.71-5 (a)(2)

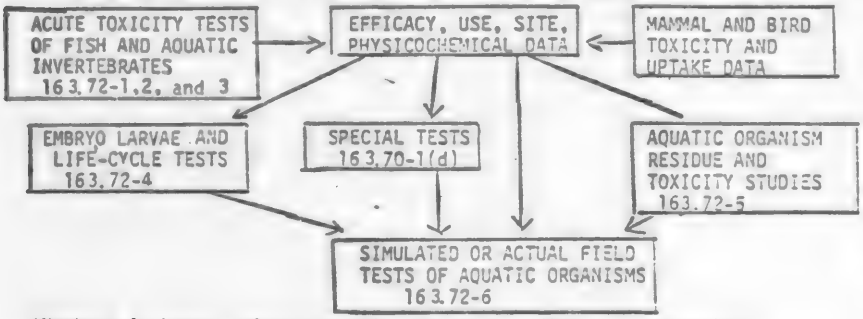
*Numbers in boxes refer to section stating conditions under which testing is required.

Figure 2 - WILD MAMMAL TESTING FLOW CHART*


SINGLE-DOSE ORAL LD50 163.71-3(a)
RELATED INFORMATION such as use pattern, chemistry, and human toxicology
MULTIPLE-DOSE 5-DAY FEEDING ORAL TOXICITY 163.71-3(c)
SPECIAL TESTS 163.70-1(d)
REPRODUCTION TESTS see human safety
SMALL PEN FIELD TEST 163.71-5 (a)(1)
LARGE PEN FIELD TEST including reproduction 163.71-5(a)(1)
FULL SCALE ENVIRONMENTAL TESTING 163.71-5 (a)(2)

*Numbers in boxes refer to section stating conditions under which testing is required.

Figure 3 - HAZARD EVALUATION TEST SEQUENCE FOR AQUATIC ORGANISMS *


ACUTE TOXICITY TESTS OF FISH AND AQUATIC INVERTEBRATES 163.72-1,2, and 3
EFFICACY, USE, SITE, PHYSICOCHEMICAL DATA
MAMMAL AND BIRD TOXICITY AND UPTAKE DATA
EMBRYO LARVAE AND LIFE-CYCLE TESTS 163.72-4
SPECIAL TESTS 163.70-1(d)
AQUATIC ORGANISM RESIDUE AND TOXICITY STUDIES 163.72-5
SIMULATED OR ACTUAL FIELD TESTS OF AQUATIC ORGANISMS 163.72-6

*Numbers in boxes refer to section stating conditions under which testing is required.

(b) *Basic standards for testing*—(1) *Personnel.* All testing and evaluation must be performed in accordance with sound experimental procedures. The Agency may require a resume of the qualifications of personnel who have performed, supervised, reviewed, or evaluated the testing.

(2) *Test methods.* (i) Toxicity tests must be conducted according to uniform methods, whenever possible, to maximize the number of reliable comparisons that can be made concerning relative toxicity and relative sensitivity.

(ii) Tests shall include control groups to determine if any observed effects have developed or occurred independent of the test substance. Positive control groups are generally not required; consult standards in specific sections of Subpart E for those tests where a positive control is required or recommended.

(iii) Control groups shall be maintained in the same manner as the test groups, provided, however, that airborne or other contamination by the test substance(s) shall be precluded.

(iv) Pen and field studies shall not be conducted in areas containing, or suspected to contain, threatened or endangered plants or animals.

(3) *Test substance.* (i) Each data requirement in succeeding sections of Subpart E specifies whether the data submitted in support of an application for registration should be derived from tests conducted with the technical grade of the active ingredient or the formulated product.

(ii) The technical grade of the active ingredient is commonly the same substance as the manufacturing-use product for which registration is sought or which is used to produce the formulated pesticide product for which registration is sought. In this case, where the sections require testing of the technical grade of the active ingredient, a sample of the manufacturing-use product shall be tested. Where this is not the case, the tests shall be conducted with the technical grade of the active ingredient which is used to produce the manufacturing-use or formulated pesticide product.

(iii) In addition to or in lieu of testing otherwise required by this subpart, the Administrator may require testing to be conducted with:

(A) An analytically pure grade of an active ingredient;

(B) The technical grade of an active ingredient;

(C) The inert ingredient of a pesticide formulation;

(D) A contaminant or impurity of an active or inert ingredient;

(E) A plant or animal metabolite or degradation product of an active or inert ingredient;

(F) The pesticide formulation; or

(G) Any additional substance which could act as a synergist to the product for which registration is sought.

(iv) The test substance shall be within the limits, if any, certified in accordance with § 163.61-6(b). The composition of the test substance shall be determined, including the name and quantities of known contaminants and impurities, so far as is technically feasible. The determination shall also include quantities of unknown materials, if any, so that 100 percent of the test sample is accounted for.

(v) The lot and sample numbers of the test substance shall be determined and recorded.

(vi) The test substance should be stored under conditions that maintain its stability.

(vii) For all long term studies, when the test substance is incorporated into feed or water, the concentration of the test substance in the feed or water shall be determined at the start of the study and samples collected periodically for analysis to verify concentrations. Certain protocols in succeeding sections of this subpart require analysis at predetermined intervals.

(viii) When the test substance is applied under simulated or actual field condition testing, residues should be determined in appropriate vegetation, soil, water, sediments, and other environmental components, and in selected tissues of test organisms.

(ix) If a carrier or vehicle is used to dissolve or dilute the test substance, it should be chosen to possess as many of the following characteristics as possible; it should not interfere with absorption, distribution, metabolism, or retention of the test substance; it should not materially alter the chemical properties of the test substance and not materially enhance, reduce, or alter the toxic characteristics of the test substance; it should not materially affect the food and water consumption of the test organisms; and at the levels used in the study, it should not produce physiological effects or have local or systemic toxicity. In addition, such a carrier or vehicle should, if possible, closely resemble, as to solvent polarity, the substance to be used under expected conditions of use.

(4) *Care and selection of test organisms.* All data submitted in support of an application for registration must be derived from tests conducted in accordance with good laboratory or field practices for handling and caring of test organisms. Only healthy organisms may be used, and they must be kept in conditions conforming to good husbandry or cultural practices. (See Animal Welfare Act, Pub. L. 94-279). Organisms should be randomly assigned to test groups to minimize bias and assure comparability of pertinent variables. The number of individuals tested per concentration and the number of concentrations or dosage levels evaluated must be sufficient to yield statistically sound data. The or-

ganisms in each test should, as nearly as practicable, be of uniform weight, size, and age, and should be phenotypically indistinguishable from wild species. Organisms selected for testing should be species of wildlife and aquatic organisms currently established in the United States and its coastal waters. In no circumstances shall threatened or endangered species be used as test organisms.

(5) *Observations.* Observations shall be made as frequently as necessary to record all signs of intoxification and abnormal behavior.

(c) *Reporting of data.* The test report shall include all information necessary to provide a complete and accurate description of test procedures and evaluation of the test results. The test report shall include a summary of the data, an analysis of the data, enough raw data for the Agency to verify calculated statistical values, and a statement of conclusions drawn from the analysis. The summary shall be of sufficient detail to permit the reader to independently understand the conclusions of the author. The metric system shall be used in test reports but the English system may also be used when appropriate. Specifically, the test report shall include all required information pertaining specifically to succeeding sections of this subpart, and the following information:

(1) *Test method.* (i) Statement of or reference to test method used and a full description of the experimental design and procedures (see also paragraph (c)(8) of this section);

(ii) The length and actual dates of the study;

(iii) The name of the laboratory performing the test, and the location where the test was performed; and

(iv) Name(s) of the principal investigator(s).

(2) *Test substance.* (i) Identification of the test substance, including chemical name, molecular structure, and qualitative and quantitative description of its chemical composition including the results of the analysis conducted in accordance with § 163.70-1(b)(3).

(ii) Manufacturer and lot and sample numbers of the test substance.

(3) *Test organisms.* (i) Identification of test organisms;

(ii) Rationale for selection of species, if species used is other than that specified or preferred in this subpart;

(iii) Age, sex, size, and weights, as applicable;

(iv) Source of supply of the organisms;

(v) Method used in assigning test organisms to test and control group; and

(vi) Description of any pre-test conditioning, including diet.

(4) *Dosing or treatment.* (i) Identification of method, route, and frequency of administration of test material, and randomization plan for treatments (when appropriate).

(ii) Rationale for selection of method, route, or frequency, if different from that recommended in this subpart;

(iii) Total volume of material administered (test substance plus carrier);

(iv) Concentrations of test substances administered (i.e., milligrams of test substance per kilogram of body weight of the organism or parts per million of the test substance in the feed or water);

(v) Dosing or treatment of control organisms; and

(vi) If the test substance is administered in the feed or water, the assay method used to determine the concentrations of the test substance.

(5) *Observations.* (i) Frequency, duration, and method of observation;

(ii) Detailed description of the nature, incidence, time of occurrence, severity, and duration of all observed toxic effects, including death and any other abnormal or unusual signs and symptoms;

(iii) Description of all abnormalities seen at gross pathological examination;

(6) *Environmental conditions*—(i) *Wildlife species (mammals and birds).* (A) A description of the current (and previous, when available) housing conditions, including number of animals per cage (see Animal Welfare Act, Pub. L. 94-279);

(B) Ambient temperature and humidity;

(C) Photoperiod and lighting;

(D) A description of the diet, including identification and composition; and

(E) Source of animal feed and water.

(ii) *Aquatic species (fish and invertebrates).* (A) A description of the test chamber conditions, including number of organisms per test chamber;

(B) The average and range of water temperature;

(C) Photoperiod and lighting;

(D) A description of the diet, including identification and composition and sources of feed;

(E) The source of dilution water, its chemical characteristics, and description of any pretreatment;

(F) Methods used for, and results of, all chemical analyses of water and all toxicant concentration determinations at beginning, during, and at end of tests, including validation studies and reagent blanks;

(G) The depth and volume of solution in aquatic test chambers;

(H) Fish standard length (where applicable);

(J) Enough LC50 values to enable determination of a toxicity curve (when applicable); and

(K) A description of the toxicant delivery system and flow rate, expressed as the average number of water volumes of test solution passing through each test chamber in 24 hours (required for flowthrough aquatic tests).

(7) *Determination of LD50 and LC50.* (i) Tabulation of the response data at each treatment level;

(ii) Calculation of the slope of the dose-response line (except that tabulation of response data will be sufficient when the method does not allow calculations of the slope);

(iii) Calculation of the LD50 or LC50, and the 95-percent confidence interval;

(iv) Methods of calculation;

(v) Estimated nondiscernible effect level; and

(vi) Statistical methods used for analyzing data.

(8) *References.* Citations of protocols used for fulfilling the requirements of the succeeding sections of this subpart, or list of references to any published literature and copy of unpublished literature used in developing the test protocol, performing the testing, making and interpreting observations, and compiling and evaluating the results shall be submitted.

(d) *Special test requirements.* In addition to the data required in all succeeding sections of this subpart, data derived from other tests may, under unusual circumstances, be required by the Agency for making judgments regarding safety to wildlife and aquatic organisms. Such data will be required when special problems are involved, and methods may usually be derived from tests already described or cited in other subparts of these guidelines. Such data requests may relate to a proposed pattern of use, a toxicological mode of action, or a unique chemical property. The data requested will be specific to the problem. Examples of such test requirements for these unusual circumstances include but are not limited to: certain chemical properties data of subpart D (§ 163.61-7); avian acute dermal LD50; certain toxicity data from subpart F, such as rabbit dermal LD50; eye irritation; acute inhalation LC50; fish or bird cholinesterase tests; metabolism; certain domestic animal safety studies mentioned in § 163.86-1; and certain effectiveness data, as required in subpart G, regarding pest control that may have major impact on the food supply or food chain of a rare or endangered species.

§ 163.70-2 Definitions. [Reserved]

AVIAN AND MAMMALIAN TESTING

§ 163.71-1 Avian single-dose oral LD50.

(a) *When required.* (1) A determination of the single-dose oral LD50 is required to support the registration of every manufacturing-use product, and of each formulated product intended for outdoor application.

(2) In cases where the LD50 is found to be in excess of 2,000 mg/kg, no calculated median response level or 95 percent confidence limits are required.

(b) *Standards.*—(1) *Substance to be tested.* The technical grade of each active ingredient in the product shall be tested.

(2) *Species.* Testing shall be performed on one avian species, either wild waterfowl (preferably the mallard) or an upland game bird (preferably the bobwhite or other native quail, or the ring-necked pheasant). The species shall be the same as one of the two species selected for the avian dietary LC50 in § 163.71-2.

(3) *Age.* Birds used in this test shall be at least 16 weeks old at the start of testing.

(4) *Controls.* A concurrent vehicle control group is required if a carrier of unknown toxicity is used.

(c) *Protocol.* The following is an example of an acceptable protocol:

METHODS AND MATERIALS

Species and history. The test species of choice are pen-reared bobwhite and mallards not less than 16 weeks of age at initiation of test. Birds should not have been mated before being placed on test. A history of rearing practices such as photoperiod, medication, and type of food should be included in the report. All lots of birds should be healthy and of uniform size, weight, and parity. All lots should be weighted, then maintained for a minimum of 15 days prior to the test. If tested indoors, temperature and humidity should be controlled, with freedom from drafts and sudden noises which could disturb the test birds. Any lots of birds that suffer an abnormal weight loss (drop of 6 percent or more) or suffer more than 10 percent mortality during the holding period should not be used.

Cages. All birds shall be caged by treatment level groups or subgroups under acceptable animal husbandry practices.

Weight. All birds must be weighed at the beginning of the test, and on days 3, 7, and 14 after administration of the test substance. Also, birds should be weighed at the termination of the test if it extends beyond 14 days.

Diet and fasting. Feed must be withheld from the control and test birds (bobwhite and mallard) for 15 hours prior to oral administration of the chemical. At all other times, birds shall have free access to feed. The diet must be standard feed ration known to be adequate for game birds and without antibiotics. Feed consumption shall be determined for treated and control birds. The feed consumed must be reported as average daily food consumption for each dose level.

Preparation of test material. 1. When a diluent is required, the preferred diluent is distilled water. However, when a test chemical is not water-soluble, other acceptable diluents include table-grade corn oil, propylene glycol, and 1-percent carboxymethylcellulose. Diluents which have known toxic or emetic properties at the level used are to be avoided. The recommended maximum amount of diluent per dosage is 0.1 to 1 percent of body weight. The total volume for all dosage levels should approximate a constant volume-to-body weight factor.

2. When preparing the test chemical for tests in which a diluent is not used, the technical material should be accurately weighed directly and put in gelatin capsules. If the chemical is first dissolved in acetone in preparation for use with capsules, the acetone should be completely evaporated at room temperature before the capsules are used.

3. A concurrent vehicle control is required if the diluent is not one of the four named immediately above. The diluent shall be administered to the control birds at the same volume as that administered to the test animals.

Methods of administration. The test chemical should be placed by oral intubation into the crop or preventriculus.

Number of birds per dose level. The number of test birds per treatment level and the number of controls shall be reported. Each number shall be no less than 10.

Dosage-mortality data. The factor between dosage levels used to calculate the acute LD50 should be based on a geometric or logarithmic scale. After preliminary screening, there should be a minimum of 5 dosage levels used in calculating the acute oral LD50. The calculation of an acute oral LD50 shall follow any acceptable method. Two examples of acceptable methods are Thompson and Weil (1952) and Litchfield and Wilcoxon (1949).

In cases where the LD50 is found to be in excess of 2,000 mg/kg, data shall be provided, based upon the 10 or more birds per dosage level, which show that less than half of the birds died at the 2,000 mg/kg dosage level. If any birds die at this level, sequentially lower levels shall be tested to provide a dose-response series which includes at least one level at which no mortality occurs.

Period of observation. The test animals shall be observed for a minimum of 14 days. The observation period shall be longer if signs of toxicity are still evident. The time of all deaths shall be recorded by day. If more than one control bird (i.e., 10 percent) dies during the test period, the test may be considered invalid, depending on the circumstances and apparent cause of the mortality.

Gross necropsy. Gross necropsies could give valuable additional data but are not necessarily required. When gross necropsies are conducted, they should be conducted on sufficient birds to characterize the gross lesions in dead birds. Gross necropsies include general inspection of the digestive tract, liver, kidneys, heart, and spleen. Any gross pathological changes shall be reported.

Recording of signs of intoxication. All signs of intoxication shall be recorded as they occur.

(d) *Reporting of data.* (1) In addition to the information specified in § 163.70-1(c), the test report shall contain the following information: dates of test; species and rearing history; source of birds; age and sex; body weights at initiation and termination of test diet used; pen dimensions; pen indoors or outdoors; weather conditions, if outdoors; temperature and humidity data, if indoors; photoperiod; feed consumption; test chemical and purity of formulation; preparation of test material; method of administration; amount of test material dosed per bird; amount of diluent dosed per bird, if used; number of birds per treatment level; number of controls employed; LD50 in mg/kg, with confidence limits; method used for calculation of LD50; length and schedule of observation period; time of mortalities; and all records of signs of intoxication or if any regurgitation occurs. The metric system should be used for units of measure.

APPENDIX

(1) Anonymous. 1968. Bobwhite quail laboratory acute oral toxicity test method (test A-2). Pesticides Regulation Division, U.S. Department of Agriculture. Washington, D.C. FR 40:26914 (June 25, 1975).

(2) Litchfield, L. J., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Therap.* 96(2):99-133.

(3) Schafer, E. W., R. B. Brunton, N. F. Lockyer, and J. W. DeGrazio. 1973. Comparative toxicity of seventeen pesticides to the quelea, house sparrow and red-winged blackbird. *Toxicol. Appl. Pharmacol.* 26:154-157 (see also page 279).

(4) Thompson, W. R., and C. S. Weil. 1952. On the construction of tables for moving average interpolation. *Biometrics* 8:51-54.

(5) Tucker, R. K., and D. G. Crabtree. 1970. Handbook of Toxicity of Pesticides to Wildlife. Fish and Wildlife Service. Resource Publication 84 (see esp. pp. 6 and 7).

(6) Tucker, R. K., and M. A. Haegele. 1971. Comparative acute oral toxicity of pesticides to six species of birds. *Toxicol. Appl. Pharmacol.* 20:57-65 (see esp. pp. 57-59).

§ 163.71-2 Avian dietary LC50.

(a) *When required.* A determination of the subacute dietary LC50 (5-day dietary exposure) is required to support the registration of all manufacturing-use products and all formulated products intended for outdoor application.

(1) In cases where the LC50 is found to be in excess of 5,000 ppm by dietary exposures, no calculated median response level or 95% confidence limits are required.

(b) *Standards.*—(1) *Substance to be tested.* The technical grade of each active ingredient in the product shall be tested.

(2) *Species.* Testing shall be performed on two avian species one species of wild waterfowl (preferably the mallard) and one species of upland game bird (preferably the bobwhite or other native quail, or the ring-necked pheasant). One of the two species selected for these studies must be the same species selected for the avian acute oral study required by § 163.71-1.

(3) *Age.* Birds used in this study should be from 10 to 17 days old at the beginning of the testing period.

(c) *Protocol.* The following is an example of an acceptable protocol:

Tests. This method determines a chemical's toxicity to birds expressed as the median lethal concentration (LC50) of chemical (technical material) in dry diet (the LC50 being the parts per million (ppm) toxicant in ad libitum diet expected to produce 50-percent mortality among birds in 8 days (5 days on treated diet followed by at least 3 days on untreated diet)).

Species. This protocol is appropriate, with modification (e.g., acclimation to test diet), for most bird species, but it addresses primarily the mallard and bobwhite.

Source of strain of birds. Test birds should be pen reared and phenotypically indistinguishable from wild birds. It is recommended that birds be utilized only from colonies that have had breeding histories maintained for them.

Age and sex. Test birds should be neither less than 10 days nor greater than 17 days of age at the initiation of the test. It should be noted that LC50 determinations may be affected by the age of the birds at the time of testing. A random selection of birds without regard to sex should be used because determination of sex is not feasible at this age.

Health of birds. Only birds in apparent good health should be used. Birds with obvious abnormalities, injuries, or sickly appearance shall be excluded. An effort should be made to precondition test birds to the test facilities and the untreated test diet for as long as possible prior to beginning of test.

Pen facilities. Pen conditions shall conform to good husbandry practices. The facilities as described in the following paragraphs are considered to be satisfactory.

(1) *Size.* Bobwhite (about 10 birds per pen) may be tested in commercial brooder units with individual pens measuring approximately 35 x 100 x 24 cm high. Waterfowl (mallards) may be tested in pens 70 x 100 x 24 cm high. Floors and external walls can be of wire mesh, while ceilings and common walls may be of galvanized sheeting.

(2) *Temperature and relative humidity.* For bobwhites and mallards, brooder temperature should be about 35° C. It can be maintained with thermostatically controlled central heating. Temperature outside the brooder may range from 22–27° C. The relative humidity should be 30–80 percent. Adequate ventilation should be maintained.

(3) *Lighting.* Fluorescent or incandescent lighting may be supplied with varying light regimes. It is preferred that the lighting be maintained continuously 24 hours per day; however, a diurnal variation is acceptable.

Test diets. A standard commercial game bird (mash form) or its equivalent should be used. Water should be continuously available. When needed to prepare test diets, the chemical may be dissolved in tablegrade corn oil or other necessary vehicle (e.g., propylene glycol) and mixed thoroughly with dry commercial mash in a ratio of 2 parts of solution to 98 parts of feed by weight. An equivalent amount of vehicle should be added to untreated diets. Diets can be mixed by commercial mechanical food mixers. Mashes and toxicant should be freshly mixed in small quantities. Then mixtures should be combined with larger quantities of mash to provide uniformity in the final dietary concentration of mash. Samples of the premix concentration shall be taken and stored for future reference and analysis.

Concentrations and dosage mortality data. Generally, each chemical should be administered in 6 dietary concentrations spaced geometrically over a span intended to produce mortality ranging from 10 to 90 percent. It is advisable to perform a range-finding study prior to selecting concentrations for testing.

Ten birds per concentration is recommended. Experimental variation may require more than 10 birds, but in no case may less than 6 birds be used.

In cases where the LC50 is found to be in excess of 5,000 ppm by dietary exposure, data shall be provided, based upon the 10 or more birds per dosage level, which show that less than half the birds died at the 5,000 ppm dosage level. If any birds die at this level, sequentially lower levels shall be tested to provide a dose-response series which includes at least one level in which no mortality occurs.

Observations on signs of intoxication. Throughout the test, all signs of intoxication and abnormal behavior shall be noted. Any unusual manifestation of the chemical to the test birds shall be recorded. The period after the 5-day exposure of birds to treated diets shall be extended for at least 3 days or for as long as test birds exhibit toxic signs and continue to die. A gross pathological examination shall be conducted on sufficient birds to characterize the gross lesions in dead birds.

Food consumption. Estimates of average food consumption per selected pens shall be made for birds in each test. Care should be taken to see that feed spillage or air contamination by volatile chemicals from pen to pen does not take place.

Statistical design. A completely randomized design should be used in each experiment with treatments and birds randomly assigned to pens.

Statistical procedure for handling data. The LC50 values and associated statistics can be derived by methods of probit analysis as described by Finney (1971) and programmed for computer by Daum and Killcreas (1966). The application of the basic probit method by Litchfield and Wilcoxon (1949), Miller and Tainter (1944), or other appropriate sources may be utilized.

(d) *Reporting of data.* (1) In addition to the information specified in § 163.70–1(c), the test report shall contain the following information: dates for beginning and ending of test; species; breeding history of test birds; source and strain of test birds; age (days); sex; body weights at initiation and termination of test; housing conditions (including temperature, relative humidity, lighting); photoperiod; test diets; test chemical; diluent(s) used; number of concentrations of chemicals used; number of birds per concentration; standard (if used); signs of intoxication (including unusual responses to chemical by test birds); results of gross necropsies; total food consumption; LC50 determinations (ppm) with 95-percent confidence limits; specific method used to calculate LD50's; and slope of dose-response line.

APPENDIX

(1) Daum, R. J. 1970. Revision of two computer programs for probit analysis. *Bull. Entomo. Soc. Am.* 16(1):10–15.

(2) Daum, R. J., and W. Killcreas. 1966. Two computer programs for probit analysis. *Bull. Entomo. Soc. Am.* 12(4):365–369.

(3) Finney, D. J. 1971. Probit Analysis, 3rd ed. Cambridge University Press.

(4) Heath, R. G., J. W. Spann, J. R. Kreitzer and C. Vance. 1970. Effects of polychlorinated biphenyls on birds. Presented at XV International Ornithological Congress, The Hague, August 30–September 5, 1970. Pp. 475–485 *in* Proceedings of the XV Int. Ornith. Congress. K. H. Voous, editor. Published by E. J. Brill: Leiden. 1972.

(5) Heath, R. G., and J. W. Spann. 1973. Reproduction and related residues in birds fed mirex. Pp. 421–435 *in* Pesticides and the Environment: A Continuing Controversy. Wm. B. Deichman, editor. Inter-Continental Medical Book Corp.: New York, N.Y. 1973. (Selected Papers, 8th Inter-Amer. Cong. on Toxicol. and Occupat. Med., University of Miami, School of Med., Miami, Fla.).

(6) Hill, E. F., R. G. Heath, J. W. Spann, and J. D. Williams. 1975. Lethal dietary toxicities of environmental pollutants to birds. U.S. Fish and Wildlife Service Special Scientific Report—Wildlife No. 191. 61 pp.

(7) Litchfield, J. T., Jr., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Therap.* 96(2):99–133.

(8) Miller, L. C., and M. L. Tainter. 1944. Estimation of ED50 and its error by means of logarithmic-probit graph paper. *Proc. Soc. Exp. Biol.* 57:261–264.

(9) Weil, C. S. 1952. Tables for convenient calculation of median-effective dose (LD50 or ED50) and instructions in their use. *Biometrics* 8:249–262.

§ 163.71–3 Mammalian acute toxicity.

(a) *When required.* Data on acute toxicity to wild mammals are required to support the registration of a formulated product when the proposed use pattern of the pesticide indicates that wild mammals may be exposed to the pesticide and the toxicity data required pursuant to subpart F are not sufficient for assessment of the potential hazard to wild mammals. Examples of such instances include but are not limited to the following:

(1) When wild ruminants would be expected to be exposed to the pesticide, and no toxicological data required under subpart F are derived from tests on ruminants.

(2) When pesticides with bactericidal properties are applied to forage of wild ruminants, and no data required under subpart F are derived from tests on sheep or cattle to indicate interference (or lack of it) with rumen fermentation.

(b) *Standards.*—(1) *Substance to be tested.* The technical grade of each active ingredient in the procuct shall be tested.

(2) *Species.* Testing shall be performed in a mammalian species representative or indicative of those found in the area(s) likely to be affected by the proposed use pattern(s).

(3) *Toxicity determination.* When the mamalian species use is large or relatively scarce, a study which determines only the approximate maximum tolerated dosage for the test species may be acceptable. In all other cases, the single-dose oral LD50 shall be determined.

(c) *Protocol.* The following is a generally acceptable protocol for toxicity studies in large and relatively scarce mammals:

INTRODUCTION

The purpose of this test for wild mammals is to detect species differences to the toxic effect of a pesticide. As such, precise LC50 values are not always essential; rather the

approximate maximum tolerated dose can be estimated and the character of the toxic syndrome determined. Careful observations and thorough necropsy examination are required. A preliminary range-finding test is first conducted to establish the approximate lethal dose, and subsequent tests with dosing of additional animals (in amounts determined according to the geometric progression indicated by the results of standard mammalian acute toxicity) are conducted to establish the approximate maximum tolerated dose. A single and/or multiple dosing schedule may be used for the test. The latter schedule, if extended for 10 days or more, can be used to indicate the relative chronicity of the pesticide in the wild species in place of an extended subacute toxicity schedule. After dosing, animals must be observed for several days for delayed toxic effects. Beyond simple mortality, signs of intoxication such as loss of weight, anorexia, and diarrhea are important. The general procedures followed in testing the toxicity of herbicides in cattle and sheep by the U.S. Department of Agriculture's Veterinary Toxicology and Entomology Research Laboratory (College Station, Tex.), Science and Education Administration (formerly Toxicological Investigation Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service) are typical and representative of common practice.

METHODS AND MATERIALS

The best policy to follow, within the constraints dictated by the particular species selected, is to exercise the following sound principles of animal selection and management. Animals must be in healthy condition, as free of social and environmental stress as possible, acclimated to diet, and uninfluenced by drugs. Normally, adults of similar age, judged at least by body weight (but preferably by additional methods as well), should be used, and data from both sexes should be obtained if possible. Use of reared stock is generally preferable to live-trapped animals.

Animal conditioning. Animals selected from appropriate sources should be allowed a prolonged period of acclimatization to the test facilities and feed. Use of replicate controls is desirable in tests with wild mammals.

Dose selection. Dosage should be calculated on a mg/kg body weight basis for the active component(s) of each chemical tested. Selection of the dosage rates for each type of test animal involves several variables. The initial dosage level should be estimated from existing acute toxicity data, and the toxic range found by trial and error. When a toxic dosage is found, additional dosages above and below this rate should then be applied to the other animals. Where a step-by-step increase of dosage indicates increased toxicity, repitition of individual dosages is not necessary.

Chemical administration. Oral dosing should be accomplished by placement in the stomach of gelatin capsules containing the test substance. A balling gun, or "drenching" (in which a solution or suspension of the chemnical is introduced into the stomach by syringe and stomach tube), may be used. Occasionally it is necessary to conceal the dosing in an attractive food item. Toxicity differences may arise from the manner of dosing. Drenching usually involves water-diluted preparations as compared to undiluted material placed in capsules. Data from the U.S. Department of Agriculture's Veterinary Toxicology and Entomology Research Laboratory indicate general inconsistency between these forms of dosing, but no generalization is possible favoring one over the other. Use of a vehicle such as corn oil and its volume may also affect the acute toxicity evaluation. The method used must be reported. For the immediate purpose of interspecies comparisons, the solvent or dispersant should match that used in the acute oral toxicity testing (§ 163.81-1). The volume administered orally should never exceed 3 percent of the body weight and should be constant at all dosage levels.

Absorption or effects related to the amount of feed in the digestive tract are minimized by overnight starvation prior to administration of the chemical dose. Feed should be provided following dosing, and water ad libitum prior to and following dosing. Withholding feed from ruminants serves little purpose; it is preferable to maintain such animals on restricted feed allotments calculated to maintain body weight.

Dosing Schedule and Test Design. Testing may use a single and/or a multiple dose schedule. If the single dose schedule is used, the test animals shall be observed for approximately 10 days following the dose for signs of toxic effect, such as loss of weight, anorexia, and abnormal function or behavior. If a multiple dose schedule is used, the test animal shall be observed during a series of 10 or more daily administrations and a minimum of 10 days following the last administered dose.

(d) *Reporting of data.* In addition to the information specified in § 163.70-1(c), test reports shall contain the following information, as appropriate, on control and treated test animals: Dates for beginning and ending test, species, source, age and how determined, body weight at initiation and termination of test, number per test, sex distribution, feed description, feed consumption, pesticide (name, type, and description), mode of administration, dose schedule, signs of toxicity (with day noted), weakness, posture, anorexia, diarrhea, irritability, mortality, accidental deaths or injuries, other signs indicated in previous mammalian tests, necropsy data, histopathology (if suggeted by observations in necropsy), estimated maximum tolerated dose, and schedule of single dose administration or repeated dose administration.

APPENDIX

(1) *Large and relatively scarce mammals.*

Agr. Res. Service U.S.D.A. Animal Disease and Parasite Research Division. 1969. The toxicity of some organic herbicides to cattle, sheep, and chickens. A.R.S. Production Research Report No. 106.

(2) *Small mammals.* The following reference provides an acceptable protocol for small mammal toxicity testing when principles of good practice set forth in the large mammal protocol are followed:

Webb, R. E. and H. Horsfall, Jr. 1967. Endrin resistance in the pine mouse. *Science* 156:1862.

§ 163.71-4 Avian reproduction.

(a) *When required.* An avian reproduction study is required to support the registration of a formulated product if any of the following conditions exist:

(1) The pesticide or any of its major matabolites or degradation products is persistent in the environment to the extent that toxic amounts on avian feed could be expected to become present under normal pesticide use situation, as determined by time and exposure product constants derived from LC50 data.

(2) The pesticide or any of its major metabolites or degradation products is stored or accumulated in plant or animal tissues, as indicated by metabolic release and retention studies (§ 163.85-1), partition coefficient for lipophilic pesticides (§ 163.61-7(c)(6)), residue tests from chronic feeding (§ 163.83-1), or structural similarity to known bioaccumulative chemicals.

(3) The pesticide product is intended for use under conditions where birds may be subjected to repeated or continued exposure to the pesticide or any of its major metabolites or degradation products, especially preceding or during the breeding season.

(4) Any other test information, such as that derived from special tests (§ 163.70-1(d)), which indicates that reproduction of birds may be adversely affected by a pesticide or by pesticide use.

(b) *Standards*—(1) *Substance to be tested.* The technical grade of each active ingredient in the product shall be tested.

(2) *Species.* Testing shall be performed on the bobwhite and mallard.

(3) *Number of test animals.* When other test data reveal bioaccumulative potential, the number of test animals in the test group should be increased sufficiently to partly offset animal deaths or data gathering problems associated with morbidity or with tissue residue determinations.

(4) *Age.* Birds approaching their first breeding season shall be used.

(5) *Dose levels.* At least two treatment level groups shall be used. A vehicle control group shall also be used. Each group shall be replicated.

(6) *Duration of administration.* Birds shall be exposed to treated diets beginning not less than 10 weeks before egg laying is expected, and extending throughout the laying season.

(c) *Protocol.* Except where noted, the following avian reproduction prototcol is acceptable for the testing of both bobwhites and mallards:

METHODS AND MATERIALS

Test animals. Pen-reared birds, previously untreated, approaching their first breeding season, and phenotypically indistinguishable from wild birds, should be used as test animals. If shipped, all birds should be examined following shipment for possible

physical injury that may have been encountered in transit. If deemed necessary, several birds may be randomly selected for pretreatment necropsy at a diagnostic laboratory to assess the state of health upon arrival. It is desirable to have a 2- to 6-week health observation period prior to selection of birds for treatment.

A history of rearing practice for the birds to be tested should be obtained if possible. This history should include lighting practices during rearing, disease record, drug and any other medication administered, and exact age.

Test groups—Bobwhites. A minimum of three test groups of bobwhites should be used. One group must serve as a control and two groups as treated birds. By random distribution, one male and two females per pen, replicated by a minimum of 12 pens, should be used per treatment group. If one male and one female per pen are used, additional pens should be added.

[10]2075

Group No.	Dosage level	Number of pens	Birds/pen	
			Male	Female
1=control	0	12	1	2
2=treated	X parts per million	12	1	2
3=treated	(n)X parts per million	12	1	2

NOTE.—(n)=some multiple.

Test groups—Mallards. A minimum of three test groups of mallards should be used. One group must serve as a control and two groups as treated birds. By random distribution, a minimum of two males and five females per pen, replicated by four or more pens, should be used per treatment group. If individual pairs (one male and one female) are to be used per pen, a minimum of 10 pens should be employed. The tables below illustrate these two examples.

Group No.	Dosage level	Minimum number of pens	Birds/pen	
			Male	Female
1=control	0	[illegible]	2	5
2=treated	X parts per million	4	2	5
3=treated	(n)X parts per million	[illegible]	[illegible]	5
1=control	0	10	1	1
2=treated	X parts per million	[illegible]	[illegible]	1
3=treated	(n)X parts per million	10	1	1

NOTE.—(n)=some multiple.

Diet preparation. Concentrations for the test substance should be based on residues expected or determined for the proposed use patterns. The concentrations should include an actual or expected field residue exposure level and a multiple level such as five. The highest nonlethal level may be estimated from data developed from the avian dietary LC50 (§ 163.71-2).

The test material should be added to table grade corn oil or other appropriate vehicle and premixed with an aliquot of basal diet, utilizing a mortar and pestle or mechanical blender. It is recommended that the aliquot of basal diet used for the premix be screened to remove large particles of diet before blending in the corn oil and test material. The final diet should be a uniformly mixed composition consisting of 98 or 99 parts by weight of the basal diet and one or two parts by weight of corn oil. The basal diet should be a commercial game bird breeder ration (or its equivalent) that is treated with an equivalent amount of vehicle. The premix should be stored frozen until utilized for preparation of the test diet. Test diets should be analyzed for pesticide concentrations at intervals during the tests. If other required long-term animal tests have demonstrated a propensity for the test chemical to persist or bioaccumulate, the degree of bioaccumulation in birds is determined by measurement of tissue residues in the birds from an extra pen group put through the reproduction test. Two or three tissues are selected for residue analysis at the end of the exposure period, based on tissues known from other studies to hold highest residues.

Testing phase—Test environment. The birds should be housed in breeding pens of adequate size conforming to good husbandry practices. The mallard pens should be screen-bottomed or kept clean of spilled food and excrement.

Since light is extremely important, both during rearing and during the egg laying period, all birds should be maintained for the first 8 weeks under a regime of 7 hours of light per day for maximum egg production.

The photoperiod should then be increased to 16-17 hours of light per day and either maintained at this level or increased by 15 minutes per week for the following 12 weeks. (The 12-week period may vary depending upon the time required for the onset of egg production). An illumination intensity of 6 footcandles at the bird level during the lighting phase of the reproductive study is adequate. Avoid the use of shorter wave-length "cool white" fluorescent lights which do not emit the daylight spectrum.

Temperature and relative humidity control throughout the reproductive test is desirable and should be recorded. Recommended levels are 21° C and 55 percent relative humidity. Ventilation is necessary.

Feeding and husbandry. All birds should receive the appropriate diet *ad libitum* for the duration of the study. Water is to be provided *ad libitum*. The test chemical should be administered for at least 10 weeks prior to the onset of egg laying.

Body weights should be recorded at weeks 0, 2, 4, 6, 8, and at the termination of treatment. During egg laying, body weight recording is discouraged because of the adverse effects that handling may have on egg production.

Food consumption should be recorded at least at bi-weekly intervals throughout the study.

Mortality should be recorded by date and morbidity (noted together with clinical signs) throughout the test phase. Gross pathology data should be obtained for birds that die during the course of the test phase and for some survivors.

Egg collection, storage, and incubation. All eggs should be collected daily, marked according to pen from which collected, and stored at 16° C and 65 percent relative humidity. Eggs should be set at weekly intervals for incubation in a commercial incubator. All eggs should be candled on day 0 for eggshell cracks; on approximately day 11 for bobwhites and day 14 for mallards to measure fertility and early death of embryos; and on day 18 for bobwhite and day 21 for mallards to measure embryo survival. For hatching, transfer of the eggs to a separate commercial incubator or hatcher should be made on day 21 for bobwhites and day 23 for mallards.

Recommended temperatures and relative humidity during hatching phase are 39° C and 70 percent, respectively.

Bobwhite chick observations. On day 24 of incubation, the hatched bobwhite chicks should be removed, hatchability recorded, chicks housed according to the appropriate parental grouping, and maintained on control diet for 14 days. The time period should be extended if mortality occurs appreciably late. The diet should be a commercial bobwhite starter diet or its equivalent.

Duckling observation. On day 27 of incubation, the hatched mallard ducklings should be removed, hatchability recorded, ducklings housed according to the appropriate parental grouping, and maintained on control diet for 14 days. The time period should be extended if mortality occurs appreciably late. The diet should be a commercial mallard starter diet or its equivalent.

Eggshell thickness. Once every 2 weeks all newly laid eggs shall be measured for eggshell thickness. The eggs laid in the first part of weeks 1, 3, 5, 7, and 9 of the egg-laying period are preferable. An accepted procedure is to crack open the eggs at the widest portion (girth or waist), wash out all egg contents, air-dry the shells for at least 48 hours, and then measure the thickness of the dried shell plus the membranes at 3 or 4 points around the girth using a micrometer calibrated to 0.01 mm units.

Analysis. Experimental groups should be compared to the control group by analysis of variance. Sample units are the individual pens within each experimental group. Analyses should include: Body weight, food consumption, eggs laid, eggs cracked, eggs set, fertility, live 3-week embryos, hatchability, number of normal chicks or ducklings, 14-day-old survivors per number of eggs hatched and per hen, and eggshell thickness.

Withdrawal. If the test substance is toxic (reduced reproduction evident), then a withdrawal study period should be added to the test phase. The withdrawal period need not

exceed 3 weeks. Continued observations should be made on egg production, fertility, hatchability, and hatching survival.

Definitions and typical observed values. The values reported here represent those observed from a few facilities under their conditions and are not necessarily representative of those from all facilities.

1. *Eggs laid.* Definitions—The total egg production during a breeding season (which is approximately 10 weeks). Normal values for bobwhites and mallards: 28 to 38 eggs per hen per season.

2. *Eggs cracked.* Definition—Eggs determined to have cracked shells when inspected with a candling lamp; fine cracks cannot be detected without utilizing a candling lamp and if undetected will bias data by adversely affecting embryo development.

Normal values for bobwhites—0.6 percent to 2.0 percent.

Normal values for Mallards—0.6 percent to 6 percent.

3. *Eggs set.* Definition—all eggs placed under incubation, i.e. total eggs laid minus cracked eggs and those selected for eggshell thickness analysis.

4. *Viable embryos (fertility).* Definition—Eggs in which fertilization has occurred and embryonic development has begun. This is determined by candling the eggs 6 to 14 days after incubation has begun. It is difficult to distinguish between the absence of fertilization and early embryonic death. This distinction can be made by breaking out eggs that appear infertile and examining further. This is especially important when a test compound induces early embryo mortality.

Normal fertility values for Bobwhites—75 percent to 90 percent.

Normal fertility values for Mallards—85 percent to 98 percent.

5. *Live 3-week embryo.* Definition—Embryo that is developing normally after 3 weeks of incubation. This is determined by candling the egg. Normal values for Bobwhites and Mallards—97 to 99 percent.

6. *Hatchability.* Definition—Embryos that mature, pip the shell, and liberate themselves from their eggs (quail on Day 23 or 24, mallard on Day 25, 26, or 27 of incubation) (computed from the number of fertile eggs). Normal values for Bobwhites and Mallards—50 percent to 90 percent.

7. *14-day-old-survivors.* Definition—Birds that survive for 2 weeks following hatch.

Nornal values for Bobwhites—75 percent to 90 percent.

Normal values for Mallards—94 percent to 99 percent.

8. *Eggshell thickness.* Definition—The thickness of the shell and the membrane of the egg at the girth after the egg has been opened and washed out, then the shell with membrane dried for at least 48 hours at room temperature.

Normal values for Bobwhites—0.20 mm to 0.22 mm.

Normal values for Mallards—0.31 mm to 0.33 mm.

(d) *Reporting of data.* In addition to the information specified in § 163.70-1(c), the test report shall contain the following information:

(1) *Test results.* The following information is to be reported for all test groups: all observed abnormal behavior, and observed morphological and physiological responses; interpretation of test conditions or results known to be unusual or anomalous shall be included.

(2) *Test conditions.* The following information is to be recorded for treated and untreated test groups: dates for beginning and ending of test; species; strain; sources; age; body weight; number per test (include sex ratio); individual identification of birds; diet; storage; feed consumption (grams per day); observations on palatability or repellency; housing conditions of test birds; space allocations for mating, nesting; protection from weather; injuries; lighting program including hours per day; wattage or footcandles at bird level; diagram of test layout; temperature; water supply; morbidity; mortality; accidental deaths or injuries; pretest and test history or medical and chemical administration; observable clinical signs; clinical tests; and post mortem autopsy.

(3) *Egg and hatching data.* The following information is to be recorded for treated and untreated test groups: Thickness; cracked eggs; eggs laid (number eggs per bird per day and per season); hatching egg storage data; temperature; humidity; days; incubation data; eggs set; egg turning frequency; fertility (viable embryos); live 3-week embryos; hatchability; pipped; dead embyros; post-hatching (juvenile birds); cripples; morbidity; mortality (14-day-old survivors); weights; and clinical signs.

(4) *Pesticide test data.* The following information is to be recorded from the test(s): application rates, levels of pesticide(s) (judge toxicant levels based on field application calculations); formulation (in corn oil) method (dietary-acute, capsule, water); analytical or bioassay method; pesticide dosage in food (ppm); pesticide dosage in bird (mg/kg/day); stability of pesticide in diet.

APPENDIX

Additional information may be found in the following references:

(1) Cochran, W. G. 1943. Analysis of variance for percentages based on unequal numbers. *Am. Stat. Assoc.* 38:287-301.

(2) Duncan. D. B. 1955. Multiple range and multiple F tests. *Biometrics* 11:1-42.

(3) Fink, R. J. 1974. Reproductive impairment evaluation parameters. From private report of Truslow Farms Inc., Chestertown, Md.

(4) Heath, R. G., J. W. Spann, and J. F. Kreitzer. 1969. Marked DDE impairment of mallard reproduction in controlled studies. *Nature* 224(5215):47-48.

(5) Kramer, C. Y. 1955. Extension of multiple range tests to groups means with unequal numbers of application. *Biometrics* 12:307-310.

(6) U.S. Department of Agriculture. 1968. A guideline for Evaluation of Reproductive Effects of Economic Poisons on Avian Species. (Handout.) Pesticides Regulation Division, Washington, D.C. (Copies available from EPA.)

(7) Davidson, K. L., and J. L. Sell. 1974. DDT thins shells of eggs from mallard ducks maintained on *ad libitum* or controlled-feeding regimes. *Arch. Environ. Contam. Toxicol.* 2(3):222-232.

(8) Heath, R. G., J. W. Spann, J. R. Kreitzer, and C. Vance. 1970. Effects of polychlorinated biphenyls on birds. Presented at the XV Internat. Ornith. Congress, The Hague, August 30–September 5, 1970. Pp. 475-485 in Proceedings of XV Internat. Ornith. Congress, K. H. Voous, editor, published by E. J. Brill, Leiden, 1973. (Selected Papers, 8th Amer. Conf. on Toxicol. and Occupat. Med., Univ. of Miami, School of Med., Miami, Fla).

(9) Heinz, G. 1974. Effects of dietary levels of methyl mercury on mallard reproduction. *Bull. Env. Cont. Toxicol.* 11:386-392.

(10) Longcore, J. R., F. B. Sampson, and T. W. Whittendale, Jr. 1971. DDE thins eggshells and lowers reproductive success of captive black ducks. *Bull. Env. Cont. Toxicol.* 6:485-490.

(11) Prince, H. H., P. B. Seigel, and G. W. Cornwell. 1968. Embryonic growth of mallard and pekin ducks. *Growth* 32:225-233.

(12) —. 1969. Incubation environment and the development of Mallard embryos. *J. Wildlife Manag.* 33:589-595.

(13) U.S. Department of Interior. 1974. Test Protocol for Mallard Reproductivity Studies at the Patuxent Wildlife Research Center. Unpublished document. September, 1974. Patuxent Wildlife Research Center, Laurel, Md. 2 pp.

(14) —. 1974. Test protocol for mallard reproductivity studies utilizing a pair of birds as the experimental unit. Unpublished document. Patuxent Wildlife Research Center, Laurel, Md. September.

§ 163.71-5 Simulated and actual field testing for mammals and birds.

(a) *When required.* The decision to require any of the following tests will be made by the Agency on a case-by-case basis:

(1) Cage or pen field tests are required to support registration of a formulated product if use of the pesticide may result in adverse effects to wildlife organisms likely to be exposed to the pesticide. Such a determination must take into account an analysis of the pesticide properties (e.g., persistence, conversion to toxic metabolites, retention on food, and repellency), intended use patterns (e.g., habitats treated, expected presence of species, and treatment amounts at toxic levels after application), and the results of other laboratory tests. More specifically, in the case of avian wildlife, the short-term (small pen) and long-term (large pen) field tests are required on the basis of the results of tests performed in accordance with the requirements of §§ 163.71-1, -2, and -4, respectively, indicating adverse effects to birds likely to be exposed to the pesticide.

(2) Full-scale field tests are required to support the registration of a formulated product if use of the pesticide may result in adverse effects to wildlife organisms likely to be exposed to the pesticide. Such determination must take into account an analysis of the pesticide properties (e.g., persistence, conversion to toxic metabolites, retention on food, and repellency), intended use patterns (e.g., habitats

treated, expected presence of species, and treatment amounts at toxic levels after application), and the results of other laboratory tests and/or cage or pen field studies demonstrating toxicity at normal use rates or evidence of long-term adverse effects.

(b) *Standards*—(1) *Basic standards.* The basic standards for conducting testing are contained in § 163.70-1(b). For full-scale field studies, a selection of non-target wildlife species shall be trapped, equipped with radiotransmitters, and tracked before, during, and after pesticide application to determine survival. The use of radiotelemetry must be an integral part of the full scale field test design, and the number of animals so tagged must be sufficient to represent a population.

(2) *Substance to be tested.* Testing shall be conducted with the formulated product.

(3) *Other standards.* Universally acceptable standards for conducting full-scale field tests are not possible because of the variety of mechanisms by which a pesticide may enter the environment, and the variety of food sources and habitats that may be affected. Therefore, the standards for conducting these tests and the information which must be reported will be established by the Agency on a case-by-case basis following consultation between the applicant and the Agency.

(c) *Protocols*—(1) *Short-term (small pen) field tests for hazard to birds.* The following is an example of an acceptable protocol for birds. Such a protocol could be adapted for use with small mammals.

This protocol has been developed as an initial simulated field test for bobwhite. With modifications, other species could be tested. This method exposes the test animals to field applications of the chemical. Exposure will not necessarily be similar to that of free-ranging animals.

METHODS AND MATERIALS

Birds. Test subjects shall be obtained from pen-reared stock. All birds shall be maintained in outdoor pens in the general area where the field test is to be conducted, for at least 2 weeks prior to being placed on test.

Each test shall include not less than six pairs of birds per test group, with one pair of birds per pen. It is recommended that at least 12 additional birds be procured and held in outdoor pens for replacement purposes.

Pens. Each pen shall contain approximately 1.8 sq m (20 sq ft) [1.2 m x 1.5 m x 0.3 m or 0.9 m x 2.1 m x 0.3 m (inside measurement)], and is to consist of 5 cm x 5 cm (2 in. x 2 in.) wooden frame covered on the inside with 1 cm (½ in.) mesh hardware cloth. Pensize may be increased to accommodate larger birds, if used. To avoid possible contamination, scrub wire and replace frames if pens have been used for previous testing. Use of aluminum tubing for framing will make cleaning of pens easier. Pen height may be increased from 30 cm (12 in.) to a height that will accommodate growth of vegetation through the test period.

Do not cover pen bottom, and stake pen securely to the ground to minimize predation. Move pens daily, or as required to maintain adequate vegetative cover. Pens should have an opening through which birds can be removed and added. Each pen is to contain a poultry waterer (preferably a 1-qt chick fount) and a small box (open on one side, 30 cm x 20 cm or 12 in. x 8 in. high) to serve as a shelter for the birds.

Test conditions. Conditions for evaluation of each pesticidal formulation should approximate those to be encountered in the use of the product. For example: evaluation of a cotton insecticide should be made in a cotton field, and the timing, rate, number, and manner of applications should be identical with those used for control of cotton insects. Similarly, products for control of grass or alfalfa insects should be tested in hayfields, pastures, or meadows. Pens of control birds are to be placed in similar habitats not subject to application of the pesticide, with subsequent handling (feeding, watering, and observation) the same as for the birds. Clearly mark all pens and all birds to facilitate accuracy in reporting.

Care should be taken at all times to avoid possible contamination due to drift from adjacent areas or from improper cleaning of equipment.

Test procedures. Pens and shelters are to be placed in position and birds (one male and one female per pen) introduced prior to application of the pesticide. Also, preceding the pesticide application, place filled waterers and approximately 85g (3 oz) of cracked corn, wheat, or other grain (scattered on the ground) in one-half of the total test and control pens used. The remaining test and control pens are to be left without feed and water for 12 hours after the pesticide application, at which time feed and water is to be introduced to these pens, as above. Sufficient food and water are to be available to the birds at all times, other than during the indicated 12-hour period.

If either member of the pair dies, the survivor is to be removed, placed in an individual holding pen, and a fresh pair placed in the pen. The survivor should be observed until death or for 14 days. Sacrifice survivors, including "control" group and birds held for replacement, at termination of the experiment.

Duration of test. For pesticides which are to be applied once per season, tests are to be continued for 14 days after the final application, with movement of pens immediately prior to each application.

(2) *Long-term (large) pen field test for evaluating hazard to birds.*

(i) The following is an example of an acceptable protocol:

INTRODUCTION

For assessing the hazards of pesticides to bobwhites and ringnecked pheasants, large cages enclosing portions of habitat of 0.005 ha (500 sq ft) or more may be utilized. With modifications other species can be also be tested by this method. Tests utilizing large pens may be conducted for pesticides to be applied on cropland, rangeland, or wildlands, or for other outdoor applications such as roadsides, rights-of-way, "waste areas," or known wildlife habitats. This type of test is not a substitute for a full-scale field study. Pesticides tested in both small pens and in the large pens may not show effects at levels as low as in actual field operations and thus experimenters may tend to underestimate the hazard to birds. This is primarily due to two reasons: (1) Bird exposure to pesticides is limited in pen situations compared with free environment where birds can, for example, feed heavily on dying invertebrates, or drink contaminated water; and (2) small, active, insectivorous and omnivorous birds are often more vulnerable to pesticide poisoning than bobwhites or ring-necked pheasants.

METHODS AND MATERIALS

Pens. Wire-covered pens should be constructed covering a minimum ground area of 0.005 ha (500 ft 2 per pen). Suitable pen dimensions for quail are 3.1 or 3.7 m by 15.2 or 22.9 m (10 ft or 12 ft x 50 ft or 75 ft) with the top cover at a height of about 2.0 m (6.5 ft). Other dimensions covering 0.005 ha or more per pen may be used. When pheasants are utilized in this protocol, the pens should be constructed so that removal of one crossfence would double the pen size; for example, two 3.7 x 22.9 m (12 ft x 75 ft) pens could be converted to one 3.7 x 45.7 m (12 ft x 150 ft) pen. Metal flashing should be placed around all pens to a height of 45.7 cm (18 in) above ground and to a depth of 15.2 cm (6 in) below the ground surface.

The number of pens needed to test one chemical is 24 to 36. This would provide 6–9 untreated (control) pens and 6–9 pens for each of 3 treatment levels (the proposed treatment rate and 2 multiples of that rate such as 3 or 5 x and 5 or 10 x). An independent water supply and a small shelter should be furnished in each pen.

Before pens are planned and constructed, wildlife agencies and successful game farms should be consulted to consider factors such as prevention of parasites and disease, soil drainage requirements, support of top cover to prevent collapse under the weight of snow, types of watering equipment, and similar information.

Birds. Pens may be stocked with one mated pair of bobwhites per pen. One-year-old birds of known history, not previously exposed to pesticides, shall be used and placed in the pens at least 2 weeks prior to the pesticide application(s). A supply of replacement bobwhites should be maintained in outdoor pens near the test pens. If either member of a test pair dies, the survivor should be removed and held for observation and a fresh pair placed in the test pen. For practical purposes, a limit should be set on the holding time and the number of replacements. If pheasants are used as the test species, the stocking rate should be 1 male and 5 females per larger pen.

Test conditions and procedures. All pens should be numbered and locations mapped or charted. Daily records should be kept of observations, toxic signs, test bird death and replacements (if any), complete data on pesticide formulations, application rates and methods, weather conditions, and all other data of value in assessing the hazard to birds. It is desirable to use movable pens that can be set up over the crop or vegetation on which the pesticide is intended to be applied. If permanent (non-portable) pens are used, then the soil should be sutiable for growing the pertinent crop or vegetation. The pesticide should be handsprayed with precision at the same rate, timing, number of applications, and formulation as the proposed registration. Replicate pens should also be treated at two multiples (such as 3 or 5 x and 5 or 10 x) of the application rate. Spraying should be done under minimum wind conditions and with protective shield-

ing to prevent contamination of adjacent sprayed pens or control pens. For statistical purposes, it is best to randomize the test pens, but because of the drift problem, it may be best to stratify the treatment pen locations.

In addition to applying the pesticide in the pens at the proposed rate and 2 multiples of that rate, feed and water should also be treated at the 3 rates. Food and water treatments should be made using procedures and rates that are consistent with the characteristics of the chemical and the usage being tested. The food treatment rates can be based on results of residue studies when such studies are requaired for other purposes in the registration procedure. Treated food (toxicants should be carried in a table gradc corn oil, not in dry form or in a repellent solvent) should be prepared within 1 day of the time the pen environments are sprayed. Treated food should be supplied to the test birds daily or every other day in feeders protected from the weather. It is also possible to provide treated animal foods such as grasshoppers or other invertebrates (earthworms, etc.) to the penned birds simultaneously with the pen environment application.

If it becomes necessary to determine the major route of pesticide exposure to the test animals, various combinations of treatments can be made. For example, treatments (with adequate replicates) might include (1) pen environment only with "clean" food and water, (2) "clean" pen environment and water with treated food only, (3) "clean" pen environment and food with treated water only, or (4) other combinations. Also, birds in half the pens at a given treatment rate may be fasted and water withheld for 12 hours prior to the pesticide application(s). If so, birds in half of the control pens should also be fasted and not allowed access to water fountains for the same 12-hour period.

Reproductive success of the test birds may also be determined. This should be done during the year of the test, and if residues of the test chemical are persistent, reporductive success of survivors should be determined one year after treatment. Eggs may be picked up periodically for artifical incubation and rearing of young in the first half of the breeding season, but eggs may be left for the hen(s) to incubate in the last half of the normal breeding season. Hens should be allowed to rear the young to 14 days of age in the test pens.

The duration of the test should be a minimum of 21 days after the final application, and longer if any birds are showing toxic signs or other effects. Reproductive tests would, of course, continue beyond 21 days post-treatment.

Pesticide residue determinations may be included in the study. Diet and water levels of the test chemicals should be analyzed. Vegetation, soil, and other environmental samples may be analyzed for residues to determine persistence and bioaccumulation. Test birds poisoned by the pesticide and a sample of surviving birds should be analyzed for residues in selected tissues. Gross pathology may be determined at the same time.

(3) *Full scale field tests for hazard to wildlife.* The primary objective of the full-scale field study is to determine the total impact of pesticide applications on wildlife populations. Effects may occur in many ways, including direct poisoning and death (by ingestion, dermal exposure, or inhalation); toxic effects indirectly causing death such as those increasing susceptibility to capture by a predator; animals' inability to reach food and water or lowering of resistence to weather and diseases; loss of insect food and supply and abandonment of home range; harmful reproductive effects and consequent inability to maintain populations; or other indirect ecological disturbances.

Full-scale field tests are useful for determing the effects of a pesticide from large-scale spraying of forests, rangelands, rights-of-way, roadsides, wetlands, and major croplands (such as cotton, wheat, corn, soybeans, rice, sorghum, or alfalfa) or other major wildlife habitats.

(i) The following may be used as guidance for conducting these tests:

METHODS AND MATERIALS

A thorough pesticide-wildlife ecology study should include collection of data on wild bird and mammal populations (resident and nonresident species), climate, soil, vegetation biomass by species, and biomass of arthropods (identified to lowest taxonomic identification possible), food habits of the most abundant wildlife species, and distribution and fate of pesticide residues in animals, plants, and environment. Each parameter or variable would require a separate sampling method. These data should be collected on intended test and control areas before and after the treatment date(s).

The experimental pesticide applications should be made on the crop(s) or type of vegetation for which the pesticide labeling makes claims for applications. Wildland treatment areas should be a minimum of 129.5 ha (320 acres) in size for a given chemical and rate of application. Cropland or right-of-way study areas may be smaller if the typical field or area sprayed would be smaller. All treatment areas should be sufficiently large to accommodate a minimum of 2 replicates of 8.1 to 16.2 ha (20 to 40 acres) census plots with sprayed buffer zone of at least 45.7 m (150 ft) around all plot boundaries. The experimental applications should be made at the proposed registration rate and at multiples of that rate (such as 3 or 5 x and 5 or 10 x). Unsprayed cropland or wildland habitat should be studied simultaneously on replicated plots in the same manner as the sprayed areas in order to detect changes due to factors other than the pesticides.

Cropland or rangeland birds may be censused by adaptations of strip method (Emlen, 1971; McEwen et al., 1972). Emphasis should be on spring and summer resident species during the breeding season. Birds in forested or mixed habitats are usually censused by plot method (Ecological Research Committee, 1970).

The basic procedure in the strip census technique is to walk a straight line transect, usually within a given time period, and to identify, record, and plot locations of all birds seen within a pre-determined width of the strip (for example, 50 m to either side of the line of travel, i.e., a width of 100 m). Strip censuses are usually run in the early morning hours to coincide with a major activity period of the birds. Transects are marked in the same manner so that the same routes can be repeated periodically over several days, weeks, months, or from year to year. Frequent counts within the breeding season on replicated transects will provide statistically adequate data for comparing pre- and post-spray populations and sprayed plots with unsprayed plots.

The general approach for plot censuses in woody or mixed habitats is similar to the strip census. The basic difference is that birds are observed, identified, and plotted on a map of a rectangular or square plot (usually about 16 ha or 40 acres in size). The observer walks a more or less set route taking him to all portions of the census plot within a given time period. Other requirements such as season, time of day, replications, and frequency of repetition, are similar to the strip method. With either method, weather conditions must be suitable for the counts.

All nests found should be marked and checked periodically with a minimum of disturbance to deterine nesting success. Reproductive success may also be estimated by recording the number of fledgings observed per unit area and time on replicated plots.

Small mammals may be studied by a mark-and-recapture method using grids of Sherman-type live traps. There are various systems of trap layouts, length of trapping period, and data treatment. The system recommended by the International Biological Program (IBP), Grassland Biome (Swift and French, 1972), utilized a square grid of 12 x 12 stations or 144 trap sites (15 m between stations) with 1 or 2 live traps per station. Animals captured are marked and released over a trapping period of 5 consecutive days. The same procedure is repeated in later time period(s) (example, pre- and post-spray) on marked, replicated grids. Data are analyzed by the Jolly (1965) method.

Effects of the pesticide treatments on total arthropod numbers and biomass should be measured in addition to effects on the target insect. These measurements can be made by standard entomological methods. Particular attention should be paid to arthropod species known to be important for wildlife food. The limitations of the arthropod sampling techniques used should be noted and reported.

Animal and environmental samples should be collected for pesticide residue determinations periodically until residue levels fall below 0.01 ppm. The following types of samples should be analyzed: (1) tissues of 1 or more species of common resident omnivorous birds, (2) tissues of 1 or more species of common resident omnivorous mammals, (3) common arthropods, (4) typical plant seeds or fruits, (5) vegetation (leaves and stems or entire above-ground parts of the wild or cultivated plants sprayed), (6) plant litter, (7) soil (to depth of about 2½ cm), and (8) water (if any) and bottom mud or silt from ponds, lakes, or streams in the drainage pattern from the sprayed ara. Much of the pesticide residue data may also be required for other registration data requirments and may be utilized for dural purposes.

(d) *Reporting of data*—(1) *Short-term (small pen) field tests.* In addition to the information specified in § 163.70–1(c), the test report shall contain the following information: test location; dates of beginning and end of test, and any other significant dates of events in the test; weather data; test animal species, source, and age; medi-

cal and chemical administration history; body weight changes; individual identification; pesticide chemical formulation, rate of application, and manner of application; vegetative cover; residue analysis; pen description; pen placement; number of animals per pen; diet; food and water supply schedule; feed consumption; visual signs of intoxication; clinical measurements for intoxication; accidental death or injuries; replacement schedule; gross pathological or histological examination results; statistical methods used.

(2) *Long term (large pen) field tests.* In addition to the information specified in § 163.70–1(c), the test report shall contain the following information:

(i) *Use and application data.* The proposed use patterns for the pesticide and any related data such as vegetation or crop(s), formulation(s), and rate(s) and date(s) of application.

(ii) *Test methods and materials data.* Test animals and their history; test pen description; test animals' food and handling; pesticide formulation(s) and application(s); statistical methods; weather data collection; analytical methods for residue determinations; clincal data methods; reproductive study methods; and any other methods utilized.

(iii) *Test results.* Mortality: number, dates, and other pertinent information; toxic signs; weight changes; food consumption; clinical observations; necropsy observations; residue analysis results; weather data and inclusive dates of test; and reproduction test results.

(iv) *Summary and conclusions.* Potential hazards to wildlife should be identified in addition to the toxicity results *per se.*

(3) *Full-scale field tests.* In addition to the information specified in § 163.70–1(c), the test report shall contain the following information:

(i) *Use and application data.* Introduction and background information including proposed use of the pesticide and related data such as vegetation or crop(s), formulation(s), and rate(s) and date(s) of application.

(ii) *Test methods and materials data.* A description of the study area including vegetation, topography, and all pertinent ecological information: study plot layout-locations and replications; listing of resident and migrant fauna with estimates of population densities or relative abundance; details of spraying equipment, methods, and weather conditions; statistical design and methods of analysis, weather data collection; analytical methods for residue determinations; clinical data methods; reproductive study methods; carcass search methods; and any other methods utilized.

(iii) *Test results.* Mortality: number, dates, and other pertinent information; signs of intoxication; bird and mammal census results; arthropod numbers and biomass; food habits data, if any; necropsy observations; residue analysis results; weather data and inclusive dates of test; and results of nest studies and fledgling wildlife.

(iv) *Summary and conclusion.* All data should be integrated into a picture reflecting the full impact on the ecology due to introduction of the pesticide. Potential hazards to wildlife should be estimated with full consideration of possible indirect effects due to ecological disturbances.

APPENDIX

(1) *Short-term (small pen) and long term (large pen) field tests.*

(i) Black, C. T., and G. L. Zorb. 1965. Effect of malathion sprays on penned pheasants. Mich. Conserv. Dept. Research and Develop. Rpt. No. 34.

(ii) Heezen, K. L. 1973. Pesticide effects on pheasants. Job Complet. Rept. Proj. No. W-118-R-6, Job No. 119.1 Mich. Dept. Conserv. 21 pp.

(iii) Kreitzer, J. F., and J. W. Spann. 1968. Mortality among bobwhites confined to a heptaclor contaminated environment. *J. Wildl. Manage.* 32(4):874–878.

(iv) Zorb, G. L. 1968. Effects of pesticides on wildlife. Job Complet. Rpt. Proj. No. W-118-R-1, Job. No. 4. Mich. Dept. Conserv. 6 p.

(2) *Full-scale field tests.*

(i) Ecological Research Committee. 1970. Recommendations for an international standard for a mapping method in bird census work. Pp. 49–52 *in* Sympos. on Bird Census and Environ. Monit., Bull. 9 (British Trust for Ornithology, Beech Grove, Tring, Hertfordshire, England).

(ii) Emlen, J. T. 1971. Population densities of birds derived from transect count. *The Auk* 88:343.

(iii) Jolly, G. M. 1965. Explicit estimates from capture-recapture data with both death and immigration—stochastic model. *Biometrika* 52:225–247.

(iv) McEwen, L. C., C. E. Knittle, and M. L. Richmond. 1972. Wildlife effects from grasshopper insecticides sprayed on short-grass range. *J. Range Manage.* 25(3):188–194.

(v) Swift, D. M., and N. R. French (Coordin.) 1972. Vertebrates-small mammals. Pp. 24–28 *in* Basic Field Data Collection Procedures for the Grassland Biome. IBP, Nat. Res. Ecol. Lab., Ft. Collins, Colo. 86 pp. (Tech. Rpt. No. 145).

AQUATIC ORGANISM TESTING

§ 163.72–1 Fish acute LC50.

(a) *When required.* A determination of the 96-hour LC50 is required to support the registration of every manufacturing-use product and of each formulated product intended for outdoor application. In addition, LC50 values at 24, 48, and 72 hours should be submitted when possible. Exceptions to this requirement are explained in paragraph (b) of this section.

(b) *Range-finding test.* Unless the approximate toxicity of the toxicant is already known, it is usually desirable to conduct a range-finding test to determine the toxicant concentrations that should be used in the definitive test. Generally, groups of five organisms should be exposed to three to five widely spaced toxicant concentrations and to control water for 24 to 96 hours using the static or flow-through techniques. The greater the similarity between the range-finding test and the required definitive test, the more useful the results of the range-finding test will be. An LC50 usually will not be determined if exposure of 30 organisms to a concentration 300,000 times the expected environmental concentration or 300 mg/l of water produces less than 50-percent mortality. Range finding data must be reported, however, when no LC50 test is reported.

(c) *Standards.*— (1) *Substance to be trested.* The technical grade of each active ingredient in the product shall be tested to support the registration of all manufacturing-use products and of all formulated products intended for outdoor application. In addition, the formulated product shall be tested if any of the following conditions exist:

(i) The product will be introduced directly into an aquatic environment when used as directed;

(ii) The LC50 of the technical grade of active ingredient approximates the expected reisdue level in the aquatic environment when the pesticide product is used as directed; or

(iii) A product component other than the active ingredient is expected to cause more than twofold enhancement of the toxicity of the active ingreditent.

(2) *Test organisms.* Testing shall be performed on one coldwater fish species, preferably rainbow trout, and one warm-water species, preferably bluegill. Very young (not yet actively feeding), spawning, or recently spent fish should not be used. Fish should weigh between 0.5 and 5.0 grams and be from the same year class. The standard length of the largest fish should be no more than twice that of the shortest fish.

(3) *Test conditions.* Water temperature should be 10–15° C. for warm-water fish.

(d) *Reporting of data.* In addition to information meeting the general reporting requirements of § 163.70–1, a report of the results of a fish acute LC50 test must include the following:

(1) Name of method, investigator, and laboratory;

(2) Dates for the period during which the test was conducted;

(3) Detailed description of test substance, including its source, composition, and additive (e.g., carriers or solvents);

(4) Detailed description of dilution water, including source, chemical characteristics (e.g., dissolved oxygen content, pH, dissolved salts), and pretreatment (if any);

(5) Detailed information about each kind of test organism, including scien-

tific name, standard length, weight, age, life stage, source, history, observed diseases, any other treatments, and acclimation procedure;

(6) Detailed description of the test, including design, containers, medium (e.g., depth and volume), treatments, way test was begun, number of organisms per treatment, loading (weight of organisms per unit volume of medium or unit surface), lighting acclimation and test temperatures (averages and range), and anything unusual about the test;

(7) Detailed descriptions of methods (or references to established methods) used for all chemical analyses of water for chemical content and toxicant concentrations, and the results of such analyses, including validation studies and reagent blanks;

(8) Detailed description of the effects of the treatment, including the criteria used to determine the effects, statement of percentages of organisms that died or showed effects of treatment, and a summary of these observations; and

(9) Any additional relevant information about the test or its results that would assist in the determination of hazard potential.

APPENDIX

(1) Examples of acceptable protocols may be found in the following reference. Fish species listed in this publication are acceptable with the exception of goldfish. Stephan, C. E., chairman. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Committee on Methods for Toxicity Tests with Aquatic Organisms. U.S. Environmental Protection Agency, Ecol. Res. Series, EPA 660/3-75-009. 61 pp.

(2) The following may be useful background information in developing acceptable protocols:

(i) Weber, C. E. (ed.) 1973. Biological field and laboratory methods for measuring the quality of surface waters and effluents. U.S. Environmental Protection Agency, Environ. Monit. Series, EPA-670/4-73-001.

(ii) Anonymous. 1975. Standard Methods for Examination of Water and Wastewater. 14th Ed. American Public Health Assoc., Washington, D.C. 1193 pp.

§ 163.72-2 Acute toxicity to aquatic invertebrates.

(a) *When required.* A determination of the EC50 or LC50 for an aquatic invertebrate species is required to support the registration of all manufacturing-use products and of all formulated products intended for outdoor application. Exceptions to this requirement are explained in paragraph (b) of this section.

(b) *Range-finding test.* Unless the approximate toxicity of the toxicant is already known, it is usually desirable to conduct a range-finding test to determine the toxicant concentrations that should be used in the definitive test. Generally, groups of five organisms should be exposed to three to five widely spaced toxicant concentrations and to control water for 24 to 96 hours using the static or flow-through techniques. The greater the similarity between the range-finding test and the required definitive test, the more useful the results of the range-finding test will be. An EC50 or LC50 usually will not be determined in exposure of 30 organisms to a concentration 300,000 times the expected environmental concentration or 300 mg/l of water produces less than 50-percent mortality or effect. Range-finding data must be reported, however, when no LC50 or EC50 test is report.

(c) *Standards.*—(1) *Substance to be tested.* The technical grade of each active ingredient in the product shall be tested to support the registration of all manufacturing-use products and of all the formulated products intended for outdoor application. In addition, the formulated product shall be tested if any of the following conditions exist:

(i) If the product will be introduced directly into an aquatic environment when used as directed;

(ii) If a product component other than the active ingredient is expected to cause more than twofold enhancement of the toxicity of the active ingredient; or

(iii) If the LC50 of the technical grade of the active ingredient approximates the expected residue level in the aquatic environment.

(2) *Test organisms.* Immature invertebrates should be used whenever possible. Among fresh-water organisms, daphnids should be in the first instar, and amphipods, stoneflies, and mayflies in an early instar.

(3) *Test conditions.* Water temperatures shall be those which are best suited to the maximum growth and health of the test organisms. For example, water temperature should be 17-21° C for daphnids, and 17°±1 C for amphipods and mayfiles (*Baetis* spp. or *Ephemerella* spp.). Water temperatures should be 12°±1 C for stoneflies. Daphnids shall be tested for 48 hours.

(d) *Reporting of data.* In addition to information meeting the general reporting requirements of § 163.70-1, a report of the results of an acute toxicity test for aquatic invertebrates must include the following:

(1) Name of method, investigator, and laboratory;

(2) Dates for the period during which the test was conducted;

(3) Detailed description of test substance, including its source, composition, and additives (e.g., carriers or solvent);

(4) Detailed description of dilution water, including source, chemical characteristics (e.g., dissolved oxygen content, pH, dissolved salts), and pretreatment (if any);

(5) Detailed information about each kind of test organism, including scientific name, weight, age, life stage, source, history, observed diseases, any other treatments, and acclimation procedure;

(6) Detailed description of the test, including design, containers, medium (e.g., depth and volume), treatments, way test was begun, number of organisms per treatment, loading (weight of organisms per unit volume of medium or unit of surface), lighting, acclimation and test temperatures (average and range), and anything unusual about the test;

(7) Detailed descriptions of methods (or references to established methods) used for chemical analyses of water for chemical content and toxicant concentrations, and the results of such analyses, including validation studies and reagent blanks;

(8) Detailed description of the effects of the treatments, including the criteria used to determine the effects, statement of pecentages of organisms that died or showed effects of treatment, and a summary of these observations; and

(9) Any additional relevant information about the test or its results that would assist in the determination of hazard potential.

APPENDIX

(1) Examples of acceptable protocols may be found in the following reference: Stephen, C. E., chairman. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Committee on Methods for Toxicity Tests with Aquatic Organisms. U.S. Environmental Protection Agency, Ecol. Res. Series, EPA 660/3-75-009. 61 pp.

(2) The following may be useful background information in developing acceptable protocols: Anonymous. 1975. Standard Methods for Examination of Water and Wastewater. 14th Ed. American Public Health Assoc., Washington, D.C. 1193 pp.

§ 163.72-3 Acute toxicity to estuarine and marine organisms.

(a) *When required.* Estuarine and marine organism toxicity tests (96-hour LC50 for shrimp, crab, and fish, and either 48-hour LC50 for embryo-larvae or 96-hour EC50 shell deposition for molluscs) are required to support the registration of a formulated product if the pesticide is intended for direct application to the estuarine or marine environment or if it may be expected to enter this environment in significant concentrations because of its expected use or mobility pattern. Exceptions to this testing requirement are explained in paragraph (b) of this section.

(b) *Range-finding test.* Unless the approximate toxicity of the toxicant is already known, it is usually desirable to conduct a range-finding test to determine the toxicant concentrations that should be used in the definitive test. Generally, groups of five organisms should be exposed to three to five

widely spaced toxicant concentrations and to control water for 24 to 96 hours using the static or flow-through techniques. The greater the similarity between the range-finding test and the required definitive test, the more useful the results of the range-finding test will be. An EC50 or LC50 usually will not be determined if exposure of 30 organisms to a concentration 300,000 times the expected environmental concentration or 300 mg/1 of water produces less than 50-percent mortality or effect. Range-finding data must be reported, however, when no LC50 or EC50 test is reported.

(c) *Standards.*—(1) *Substance to be tested.* The technical grade of each active ingredient in the product shall be tested. In addition, tests shall be performed with the formulated product if any of the following conditions exist:

(i) The product will be introduced directly into an aquatic environment when used as directed;

(ii) The LC50 of the technical grade of active ingredient approximates the expected residue level in the aquatic environment when used as directed; or

(iii) A product component other than the active ingredient is expected to cause more than twofold enhancement of the toxicity of the active ingredient.

(2) *Test organisms and test duration.* The 96-hour LC50 shall be determined for shrimp, crab, and an estuarine or marine fish. The 48-hour LC50 for oyster embryo-larvae or 96-hour EC50 shell deposition data shall be determined on a representative mollusc, such as the American oyster.

(d) *Reporting of data.* In addition to information meeting the general reporting requirements of § 163.70-1, a report of the results of an acute toxicity test for estuarine and marine organisms must include the following:

(1) Name of method, investigator, and laboratory;

(2) Dates for the period during which the test was conducted;

(3) Detailed description of test substance, including its source, composition, and additives (e.g., carriers or solvent);

(4) Detailed description of dilution water, including source, chemical characteristics (e.g., dissolved oxygen content, pH, dissolved salts), and pretreatment (if any);

(5) Detailed information about each kind of organism, including scientific name, weight, age, life stage, source, history, observed diseases, any other treatments, and acclimation procedure;

(6) Detailed description of the test, including design, containers, medium (e.g., depth and volume), treatment, way test was begun, number of organisms per treatment, loading (weight of organisms per unit volume of medium or unit of surface), lighting, acclimation and test temperatures (average and range), and anything unusual about the test;

(7) Detailed descriptions of methods (or references to established methods) used for all chemical analyses of water for chemical content and toxicant concentrations, and the results of such analyses, including validation studies and reagent blanks;

(8) Detailed description of the effects of the treatment, including the criteria used to determine the effects, statement of percentages of organisms that died or showed effects of treatment, and a summary of these observations; and

(9) Any additional relevant information about the test or its results that would assist in the determination of hazard potential.

APPENDIX

(1) *Acute estuarine and marine fish, crab, and shrimp toxicity.* Examples of acceptable protocols may be found in the following references:

(i) Bahner, L. H., C. D. Craft, and D. R. Nimmo. 1975. A salt-water flow-through bioassay method with controlled temperature and salinity. *Prog. Fish-Cult.* 37(3):126–129.

(ii) Stephan, C. E., chairman. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Committee on Methods for Toxicity Tests with Aquatic Organisms. U.S. Environmental Protection Agency, Ecol. Res. Series, EPA 660/3-75-009. 61 pp. (Marine and estuarine species listed in this publication are acceptable.)

(2) *Marine mollusc shell deposition and embryo-larvae toxicity tests.* Examples of acceptable protocols may be found in the following publications:

(i) Anonymous. 1975. Standard Methods for the Examination of Water and Wastewater. 14th Ed. American Public Health Association, New York. 1193 pp.

(ii) Woekle, C. E. 1967. Measurements of water quality with the Pacific oyster embryo bioassay. Pp. 112-120 *in* Water Quality Criteria, ASTM, STP 416. American Society for Testing and Materials, Philadelphia, Pa.

(3) *General.* The following references may aid in the performance of marine toxicity testing.

(i) Clark, J. R., and R. L. Clark, eds. 1964. Seawater systems for experimental aquariums. U.S. Dept. Int., Fish. and Wild. Serv. Bur. Sport. Fish. Wild. Res. Rep. No. 63, 192 pp.

(ii) DeBen, E. A. 1970. Design and construction of saltwater environment simulator. Fed. Water Qual. Admin., Pacific N.W. Water Lab., Working Paper 71:1-30.

(iii) Shelbourne, H. E. 1962. Experimental seawater systems for rearing fish larvae. Pp. 81-93 *in* J. R. Clark and R. L. Clark, eds. Seawater systems for experimental aquariums. U.S. Dept. Int., Fish. Wild. Serv. Bur. Sport Fish. Wild. Res. Rep. No. 63, 192 pp.

(iv) Strickland, J. D. H., and T. R. Parsons. 1968. A practical handbook of seawater analysis. Fish. Res. Board Can. Bull. No. 167, 311 pp.

(v) White, D. B., R. R. Stickney, D. Miller, and L. H. Knight. 1973. Seawater systems for aquaculture of estuarine organisms at the Skidaway Institute of Oceanography. Ga. Mar. Sci. Center, Technical Rep. Ser. No. 73-1. 18 pp.

(vi) Wood, L. 1975. A controlled condition system (CCS) for continuously flowing seawater. *Limnol. Oceanogr.* 10:475-477.

(vii) Anonymous. 1978. Bioassay Procedures for the Ocean Disposal Permit Program. U.S. Environmental Protection Agency, Office of Research and Development. EPA-600/9-78-010. 121 pp.

§ 163.72-4 Embryolarvae and life-cycle studies of fish and aquatic invertebrates.

(a) *When required.* Embryolarvae or life-cycle tests (or both) are required to support the registration of a formulated product if the pesticide product is used in or is expected to transport to water from the intended use site, and if the following considerations apply:

(1) *Fish embryolarvae or invertibrate life-cycle test.* (i) If the LC50 is less than 1 mg/l;

(ii) If estimated concentration in water is greater than 0.01 of the LC50;

(iii) If actual or estimated concentrations in water resulting from use are less than 0.01 of the LC50; and

(A) Reproduction is significantly affected as demonstrated by mammalian or avian studies of systems relevant to fish or invertebrates,

(B) Physicochemical properties indicate cumulative effects, or

(C) The pesticide is persistent in water (e.g., one-half life in water greater than 4 days); or

(iv) If the pesticide is intended for broad use such that it is likely to be present in water continuously, regardless of properties.

(2) *Fish life-cycle test.* (i) If adverse effects are observed in fish embryolarvae or invertebrate life-cycle test;

(ii) If the estimated environmental concentration is one-tenth of the "no effect" level observed in the fish embryolarvae or invertebrate life-cycle test; or

(iii) If significant effects relevant to fish reproduction are observed in mammalian or avian reproduction studies at relatively low concentrations.

(b) *Standards*—(1) *Substance to be tested.* The technical grade of each active ingredient in the product shall be tested.

(2) *Duration of tests*—(i) *Aquatic embryolarvae test.* The embryolarvae test requires that aquatic organisms be exposed to the test substance through the embryolarvae phase (e.g., a fish "egg-fry" test) but not all stages of life-cycle of one generation of the species.

(ii) *Aquatic life-cycle tests.* The life-cycle test requires that aquatic organisms be cultured in the presence of the test substance from egg to egg or from one stage of life cycle to the same stage of the next generation

(3) *Species.* Tests with live-bearing fishes are not acceptable.

(i) *Fish life-cycle test.* Testing should be performed on a fresh water fish, preferably the fathead minnow. An estuarince species (e.g., sheepshead minnow) may be used instead if the pesticide may contaminate the estuarine environment.

(ii) *Reproduction and other embryo-larvae and life-cycle tests.* The applicant shall consult with the Agency regarding appropriate species and test methodologies.

(c) *Reporting.* In addition to the basic information specified in § 163.70-1(c), the test report shall contain the following information (where appropriate): reproductive effects; detailed records of spawning, egg numbers, fertility, and fecundity; estimated nondiscernible effect level, and mortality data; statistical evaluation of effects; locomotion, behavioral, physiological, and pathological effects; definition of the criteria used to determine effects; summary of general observations of other effects or signs of intoxication; and stage of life cycle in which organisms were tested.

APPENDIX

(1) *Aquatic embryo-larvae test.* Examples of acceptable protocols may be found in the following references:

(i) National Water Quality Laboratory Committee on Aquatic Bioassays. 1971. Recommended bioassay procedure for fathead minnow *Pimephales promelas* (Rafinesque) chronic tests. National Water Quality Laboratory, Duluth, Minn. 13 pp. (Revised January 1972.)

(ii) ——— 1971. Recommended bioassay procedure for brook trout *Salvelinus fontinalis* (Mitchell) partial chronic tests. National Water Quality Laboratory, Duluth, Minn. 11 pp. (Revised January 1972.)

(iii) Hansen, D. J., P. R. Parrish, S. C. Schimmel, and L. R. Goodman. 1978. Life-cycle toxicity test using sheepshead minnows (*Cyprinodon variegatus*). Pp. 109-116 in Bioassay Procedures for the Ocean Disposal Permit Program. U.S. Environmental Protection Agency, Office of Res. and Dev. EPA-600/9-78-010.

(2) *Aquatic life-cycle test.* (i) *Freshwater fish life-cycle test.* Examples of acceptable protocols may be found in the following reference: National Water Quality Laboratory Committee on Aquatic Bioassays. 1971. Recommended bioassay procedure for fathead minnow *Pimephales promelas* (Rafinesque) chronic tests. National Water Quality Laboratory, Duluth, Minn. 13 pp. (Revised January 1972.)

(ii) *Estuarine fish life-cycle test.* Examples of acceptable protocols may be found in the following references:

(A) Schimmel, S. C., and D. J. Hansen. 1974. Sheepshead minnow *Cyprinodon variegatus:* an estuarine fish suitable for chronic (entire life-cycle) bioassays. *Proc. 28th Ann. Conf. S.E. Assoc. Game-Fish Comm.* pp. 392-398.

(B) Hansen, D. J., P. R. Parrish, S. C. Schimmel, and L. R. Goodman. 1978. Life cycle toxicity test using sheepshead minnows (*Cyprinodon variegatus*). Pp. 109-116 in Bioassay Procedures for the Ocean Disposal Permit Program. U.S. Environmental Protection Agency, Office of Res. and Dev. EPA-600/9-78-010.

(iii) *Invertebrate life-cycle test.* Examples of acceptable protocols may be found in the following references:

(A) Biesinger, K. E. 1974(a). Procedure for *Daphnia magna* tests in standing system. U.S. Environmental Protection Agency, Environ. Res. Lab., Duluth, Minn.

(B) Biesinger, K. E. 1974(b). Procedure for *Daphnia magna* chronic tests in flowing system. U.S. Environmental Protection Agency, Environ. Res. Lab., Duluth, Minn.

(C) Nimmo, D. E., T. L. Hamaker, and C. A. Sommers. 1978. Entire life-cycle toxicity test using mysids (*Mysidopsis bahia*) in flowing water. Pp. 64-68 in Bioassay Procedures for the Ocean Disposal Permit Program. U.S. Environmental Protection Agency, Office of Res. and Dev. EPA-600/9-78-010.

(iv) *Additional information.* Additional information may be found in the following reference: Biesinger, K. E. 1974(c). Culturing methods for *Daphnia* and certain other cladocerans. U.S. Environmental Protection Agency, Environ. Res. Lab., Duluth, Minn.

§ 163.72-5 Aquatic organism toxicity and residue studies.

(a) *When required.* Aquatic ecosystem testing is required to support the registration of all formulated products if, after an analysis of the pesticide properties, the individual use patterns, and the results of previous tests, it is determined that use of a pesticide may result in adverse effects on the nontarget organisms in aquatic environments, including those of the water column and bottom sediments. Specific requirements will be established on a case-by-case basis. When a pesticide is used in or is expected to transport to water from the intended use site, major considerations for requiring these toxicity and residue tests include, but are not limited to:

(1) Physico-chemical data indicating accumulation (e.g., water solubility less than 0.5 mg/1 and octanol/water partition coefficient greater than 1000), and (2) persistence in water (e.g., half-life in water greater than 4 days), or (3) accumulation in organs and tissues, as indicated by mammalian or avian studies. For residue data on fish, the requirements of § 163.62-11(d), subpart D, of these guidelines shall apply. Requirements for residue studies involving other food chain organisms will be established on a case-by-case basis.

(b) *Standards*—(1) *Substance to be tested.* The technical grade of each active ingredient in the product shall be tested.

(2) *Test organisms.* Toxicity and residue studies may be required using a typical bottom-feeding species (e.g., channel catfish or carp); predator fish representative of cold-water or warm-water habitats (e.g., brook or rainbow trout, and bass, bluegill, northern pike or walleye); molluscs (e.g., oysters or freshwater clams); crustaceans (e.g., *Daphnia* spp., *Gammarus* spp., or crayfish); or nymphs (e.g., mayfly).

APPENDIX

Some acceptable protocols may be developed from the following references:

(1) Johnson, B. T., and R. A. Schoettger. 1975. A biological model for estimating the uptake, transfer, and degradation of xenobiotics in an aquatic food chain. *Fed. Regis.* 40 (123): 26906-26909. (June 25, 1975.)

(2) Macek, K. J., M. E. Barrows, R. F. Frasny, and B. H. Sleight, III. 1975. Bioconcentration of ^{14}C-pesticides by bluegill sunfish during continuous exposure. Pp. 119-142 in G. D. Veith and D. E. Konasewich, Eds. Structure-activity correlations in studies of toxicity and bioconcentration with aquatic organisms. Proceedings of a Symposium, Burlington, Ontario, March 11-13, 1975. Sponsored by Standing Committee on Scientific Basis for Water Quality Criteria of the International Joint Commission's Research Advisory Board.

(3) Schimmel, S. C., J. M. Patrick Jr., and A. J. Wilson. 1977. Acute toxicity to and bioconcentration of endosulfan by estuarine animals. Pp. 241-252 in F. L. Mayer and J. L. Hamelink, Eds. Aquatic Toxicology and Hazard Evaluation. STP No. 634, American Society for Testing and Materials, Philadelphia, Pa.

§ 163.72-6 Simulated or actual field testing for aquatic organisms.

(a) *When required.* (1) A short-term simulated field test (where confined populations are observed), or an actual short-term field test (where natural populations are observed), or both, are required if laboratory data indicate adverse short-term or acute effects may result from intended use.

(2) A long-term simulated field test (e.g., where reproduction and growth of confined populations are observed) or an actual field test (e.g., where reproduction and growth of natural populations are observed), or both, are required if laboratory data indicate adverse long-term, cumulative, or life-cycle effects may result from intended use.

(b) *Standards*—(1) *Basic standards.* Basic standards for conducting testing are contained in § 163.70-1(b).

(2) *Substance to be tested.* These studies are to be conducted with the formulated product.

(3) *Other standards.* Universally acceptable standards for conducting field tests are not possible because of the many mechanisms by which a pesticide may enter the environment, and the variety of food sources and habitats that may be affected. Therefore, the standards for conducting these tests and the information which must be reported will be established by the Agency on a case-by-case basis following consultation between the applicant and the agency.

APPENDIX

Some acceptable protocols may be developed from the following reference:

Anonymous. 1975. Field testing techniques. *Fed. Regis.* 40(123):26909-26912. (June 25, 1975.)

ADDENDUM I

FIFRA section 25(a)(2)(A) requires the Administrator to provide the Secretary of Agriculture with a copy of any proposed regulation at least 60 days prior to signing it for publication in the FEDERAL REGISTER. This provision is to permit the Secretary opportunity to comment on the proposal, and he did so for these guidelines and preamble on subparts B, D, and E. The comments of the Secretary and the Administrator's response thereto are published below. In this instance, correspondence was exchanged between Edwin L. Johnson, Deputy Assistant Administrator for Pesticide Programs, EPA, and Philip C. Kearney, Ph.D., Acting Pesticide Coordinator, Office of Environmental Quality Activities in the Office of the Secretary, U.S. Department of Agriculture. Following the introductory paragraph from Dr. Kearney, each of the 30 relatively short individual comments is reproduced in full. The page numbers mentioned in Dr. Kearney's letter refer to pagination in the draft (dated Jan. 30, 1978) he was reviewing. For purposes of brevity in this appendix, each USDA comment is followed immediately by insertion of the corresponding EPA response printed in italics and set off in brackets. Readers are reminded that comments referring to guidelines sections beginning "162" should be interpreted to be those that are now "163" in the proposed guidelines.

DEPARTMENT OF AGRICULTURE,
OFFICE OF THE SECRETARY,
Washington, D.C., March 13, 1978.

MR. EDWIN L. JOHNSON (WH-566),
Deputy Assistant Administrator for Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C.

DEAR MR. JOHNSON: We are pleased to comment on the "Proposed Guidelines for Registering Pesticides in the United States": Subpart B. Introduction; Subpart D. Chemistry Requirements; and Subpart E. Hazard Evaluation: Wildlife and Aquatic Organisms."

Following are comments applicable to the "Chemistry Requirements" as reviewed by the Department.

Effect of Microbes on Pesticides (p. 35-36, referring to §162.62-8(b)(c). The requirement for identifying genera responsible for pesticide degradation seems unreasonable. Isolation of pure cultures is useful when examining detailed pathways of pesticide metabolism, yet these species may not necessarily be important contributors to pesticide breakdown in the immensely complex whole soil milieu. Furthermore, an organism may (as with the pyrethroid insecticides) cause certain molecular changes, yet be incapable of that prerequisite initial modification of the pesticide. What then, is the significance of identifying these secondary organisms? Finally, there are examples or organisms, known to degrade pesticides in laboratory tests, which are not themselves found in normal agricultural soils. How much importance shall we give to these organisms, or the environmental stresses that affect their growth?

[EPA response: *The Agency recognizes the limitations that must be applied to interpretations of data regarding the kinds of microbes that can contribute to pesticide breakdown. However, failure to gain any knowledge about this subject would be naive when pesticides are approved or recommended for use in great quantities annually on soil or crops grown in soil. This subject needs considerably further study, and the Agency is hopeful that superior requirements with more applicability will be forthcoming, some possibly from USDA. Constructive public comments may also lead us in the right direction. Wording in the preamble was modified slightly to reflect no more stringent requirements than the corresponding guidelines.*]

Effect of Microbes on Pesticides (p. 35-36, referring to §162.62-8(b)(c). It still seems most reasonable to emphasize only the persistence and products of breakdown of the pesticide, under a range of conditions related to the chemical's intended use. Sterilization techniques can lead to misleading interpretations (such as with amitrole, in which the chemical degradation is inhibited by sterilization). Thus, even this requirement (nonsterile *and* sterile incubation) is unwarranted.

[EPA response: *As stated in the preamble, industry comments already received suggest that the soil metabolism studies be modified to include sterile and nonsterile conditions, and the suggestion was adopted. The Agency recognizes the limitations that must be applied to interpretations of these data, but recommends further public comment before considering changes or deletions of any of these requirements.*]

Effect of Pesticides on Microorganisms (p. 36-37, referring to §162.62-8(f)(3). Some chemicals are added for the purpose of controlling soil microorganisms, e.g., fungicides, fumigants, and the newer inhibitors of nitrification or degradation of specific pesticides. In general, however, chemicals have little long-term impact on soil biota. Where changes occur, they are more likely to be confined to a narrow surface layer of soil containing the pesticides. Adverse effect is likely to be quickly overcome as crops derive their nutrients from deeper soil zones.

Soil microorganisms as a whole seem to be self-reliant (enzymatically). Thus, elimination or reduction of sensitive species tends to trigger a sequence of replacements within the population, resulting in the preservation of metabolic integrity. We recommend that regulatory requirements be aimed exclusively at important processes (e.g., O_2 consumption, CO_2 evolution, N-cycle reactions, cellulose decomposition, and/or measurement of enzyme activity, such as dehydrogenase or phosphatase). It should always be remembered that temporary inhibition of one or more of these processes is not necessarily a negative effect, as population may recover within days or weeks. Perhaps only long-lasting effects (months+) need special consideration.

[EPA response: *The Agency agrees with the suggestions of aiming the data requirements toward the named important processes, and has therefore incorporated the suggestion. However, such data should not be exclusive of the requirements already set forth in the guidelines, and therefore no deletion of current proposed requirements was made. The Agency also agrees that evidence of temporary vs. long-lasting effects be demonstrated, and incorporated appropriate language into the guidelines reflecting this requirement.*]

Bound Residue Data (p. 39-40, referring to §162.79(a)(2)(vi) of the 1975 guidelines). The term "unextractable" residue is perhaps better described as "unextracted" residue. Continued exploration of solvent systems and extraction techniques may well release more residue.

[EPA response: *"Unextractable" as used in the guidelines was intended to mean "in keeping with current scientific knowledge and methodology". However, since this subject is about a discontinued requirement anyhow, the Agency sees no reason for further modification.*]

Specific Reporting Requirements—Adsorption Data (p. 91). Change the first sentence to read: "Adsorption data must include the soil: solution ratio as well as the concentration of pesticide in each phase."

[EPA response: *The suggestion was adopted by adding the phrase "and a calculation of the soil/solution ratio" to the end of the sentence.*

Leaching (p. 105-106). Reference to "twenty acre-inches" and "one-half acre inch" water per day are not in the required metric format. Furthermore, the rationale for 20" of water is not offered, nor is this amount related in any way to soil column length. If a chemical largely disappears from a short column, how can predictions of depth of leaching be made for either a longer column or the field? The 20" of water is not correlated with soil TLC data (the alternative method) *unless* investigators using the column can provide profiles of both water and pesticide movement, preferably in a long column.

[EPA response: *Conversion to metric equivalents for 20 acre inches and ½ acre-inch was made and incorporated in the text. Also, the column height is now stated in centimeters. To date, the Agency has not been consistent regarding the requirement of a specific column length for leaching data, and now concurs with the USDA suggestion of a long column; therefore, the column length of 30 centimeters has been incorporated in these proposed guidelines.*]

Following are the comments applicable to the "Hazard Evaluation: Wildlife and Aquatic Organisms" as reviewed by the Department.

Testing Requirements for TSCA versus Testing Requirements to meet FIFRA (p. 6). Any data requirements for parent compounds or constituents in the formation of a pesticide should be similar to those of FIFRA so that data derived to meet TSCA regulations can be used to meet FIFRA regulations and, therefore, not require duplicative studies.

[EPA response: *The Agency agrees with this comment, and Agency staff members operating under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Toxic Substances Control Act (TSCA) are working closely together to see that no unnecessary duplications take place.*]

Appropriateness of Extending Environmental Chemistry Data Requirements to "Manufacturing Use Products" (p. 10). Any extension of such requirements to include manufacturing-use products would prove costly. Toxicity data would be able to show effects, or lack of effects, at levels which can be detected in the chemical analysis. Otherwise, the data will not benefit the decision making process.

[EPA response: *To reduce misinterpretation of the intent of the Agency, the sentence in question has been modified as follows: "Consequently, the environmental chemistry data requirements reflect the Agency's greater concern with the environmental fate and movement of formulated products."*]

The sentence beginning, "Generally, all data requirements must be satisfied. . . ." (p. 12). The word "satisfied" should be followed by "or waived."

[EPA response: *The word "generally" at the beginning of the sentence is sufficent to*

cover exceptions by waivers or other means.]

Section E. Retention and Submission or Raw Data, Specimens and Samples of Test Substances (p. 19). The exacting conditions required for maintaining raw data and associated specimens and samples for unspecified periods of time may require an unnecessary drain on limited resources.

[EPA response: *The Agency is requesting comment on its economic impact analysis which states that costs associated with this requirement are deemed negligible.]*

Section IV, Part A. Selection of Avian Species (p. 44). The mandatory requirement of "wild" test species may be in direct conflict with both the State Game Codes and the purpose of such laws. Captive reared specimens of the game species should give comparable results. The use of domestic fowl may also provide useful information, especially where such species may be exposed.

[EPA response: *The Agency intended that captive reared specimens of the game species mentioned should be used, and has incorporated the words "captive reared specimens" into the wording.]*

Scope and Intent (p. 52, referring to § 162.40-1). The entire section could begin with Item C on page 53.

[EPA response: *The Agency disagrees with this statement; the legal basis for the guidelines and reason for registration data requirements are important and should remain in the document.]*

Deviation from Test Standards and Acceptable Protocols (p. 58, referring to § 162.40-3(a)(1)(ii)). In the second sentence delete the words "if any".

[EPA response: *The Agency agrees that the words "if any" are not useful in the sentence, and deleted them.]*

Deviation from Test Standards and Acceptable Protocols (p. 58, referring to §162.40-3(a)(2)). This statement should consider that data unacceptable for registration due to the protocol followed is also unacceptable for regulatory purposes for the same reason.

[EPA response: *The Administrator does not agree with this comment. Data which deviates from the standards contained in the guidelines would be acceptable for registration and other regulatory purposes under Section 162.40-3. Data must be reliable for the purpose for which it is being used. Data which does not meet the standards of the guidelines but is otherwise reliable and valid according to the requirements for valid tests as recognized by experts will be used, as appropriate, for regulatory purposes. [See Section 162.11(a)(3) of the registration regulations.] Also, certain kinds of data, such as benefits information, submitted as rebuttal to certain presumptions against registration [§ 162.11(a)] may not necessarily conform to the usual registration requirements.]*

Retention and Submission of Raw Data, Specimens, and Samples of Test Substances (p. 58, referring to § 162.40-5). The requirement for the maintenance of archives, storage, etc. could exceed all practical value in terms of maintenance time requirements.

[EPA response: *EPA expects that retaining such material may be of significant importance, particularly in view of the Agency's lab audit program which is being conducted in cooperation with the Food and Drug Administration of DHEW. Once such material is labeled, cataloged, and stored, the maintenance burdens should be negligible.]*

General Requirements (p. 139, referring to § 162.70-1(b)). The words "as recognized by the appropriate scientific community" should be deleted unless properly defined.

[EPA response: *The Agency agrees that the phrase "as recognized by the appropriate scientific community" adds little to the usefulness of the sentence, and it is therefore deleted.]*

General Requirements (p. 139, referring to § 162.70-1(b)(i)). The words "to similar test organisms" are suggested for addition to the end of the sentence.

[EPA response: *The Agency does not agree that the addition would be useful as suggested, and has not modified the sentence; to do so would introduce unnecessary confusion.]*

Avian Single-dose Oral LD50 (p. 147-148, referring to § 162.71-1). The requirements of pen-reared birds in the test protocol is inconsistent with the requirement for "wild" species (page 44).

[EPA response: *Since the wording was corrected per the earlier comment, there is no longer an inconsistency.]*

Avian Single-dose Oral LD50 (p. 149, referring to § 162.71-1, Preparation of Test Material, item 2). The first sentence is not clear.

[EPA response: *The word "microsyringed" was replaced by the words "put in", so that the test chemical could be solid or liquid; presumably this clarifies the sentence.]*

Avian Single-dose Oral LD50 (p. 150, referring to § 162.71-1, Dosage-mortality data). The statistical method used should be reported.

[EPA response: *The statistical method used is required to be reported, as stated in Section 162.71-2 (d) on reporting of data.]*

Avian Dietary LC50 (p. 153, referring to § 162.71-2, Species). It is felt the protocol is neither appropriate for insectivorous birds nor acceptable for grainivorous birds without first acclimating the birds to accept the diets normally used (mash form) in these tests. This would require behavioral modification prior to conducting such tests.

[EPA response: *The Agency agrees with the comment, and has added, after "modification", the phrase "(e.g., acclimation to test diet)".]*

Avian Dietary LC50 (p. 153, referring to § 162.71-2, Age and Sex). It is suggested the ages of these birds should be comparable to the ages required in the LD_{50} studies.

[EPA response: *The Agency disagrees with making a change at this time. The age of birds for the various tests is a subject of considerable continuing discussion. The preamble (IV(c)) discusses some of the aspects of the subject.]*

Avian Dietary LC50 (p. 156, referring to § 162.71-2, Statistical Procedure for Handling Data). The reference to Finney (1952) should be updated to the 3rd edition published in 1971. In general, this section needs updating.

[EPA response: *The Agency agrees that the more recent reference should be cited, and has made the appropriate corrections in the text and appendix.]*

Mammalian Acute Toxicity (p. 158, referring to § 162.71-3). The requirement as presented may fall in the "nice to know" category and not provide very useful information.

[EPA response: *These data are rarely required because most information on toxicity to mammals will come from lab animal tests (now delineated in Subpart F of these guidelines). However, there are times when such data on certain species are needed, and the purpose of this section is to obtain such data when required.]*

Avian Reproduction (p. 164, referring to § 162.71-4(a)(1)). The entire statement is not clear. Section 162.71-4, Avian Reproduction (a)(1)(2)(3)(4) covers nearly all pesticide application conditions and thus it appears an avian reproductive study would become mandatory for all pesticides. Is this the intent of the Agency?

[EPA response: *Requirements to submit these data are currently rare, and under the proposed guidelines the intent has also been that the data be required only occasionally. The Agency is inviting public comments to suggest better criteria to state when such data should be submitted. See preamble (IV(E)).]*

Simulated and Actual Field Conditioning Testing for Mammals and Birds (p. 176 and 177, referring to § 162.71-5(a) (1) and (2)). These paragraphs are sufficiently vague to indicate that such testing could be required for all pesticides to be applied out-of-doors. Is this the intent of the Agency?

[EPA response: *Same response as immediately above.]*

[*Simulated and Actual Field Conditioning Testing for Mammals and Birds* (p. 177, referring to § 162.71-5(b)(1).] The use of radiotelemetry on a selection of nontarget species alone is not likely to provide useful information. The use of such techniques must be an integral part of the test design and the number of animals so tagged must be sufficient to represent a population.

[EPA response: *The Agency agrees with the intent of the comment, and has therefore incorporated appropriate language in the paragraph to meet this intent: "The use of radiotelemetry must be an integral part of the test design, and the number of animals so tagged must be sufficient to represent a population."]*

Simulated and Actual Field Conditioning Testing for Mammals and Birds (p. 177, referring to § 162.71-5(1)(c) (1) and (2), Methods and Materials). This protocol requires field caged birds for test purposes while there have been indications such tests are unsatisfactory.

[EPA response: *EPA is not aware of any firm evidence indicating that tests with field-caged birds are unsatisfactory. Therefore, the Agency feels no change or comment is necessary.]*

Acute Toxicity to Aquatic Invertebrates (p. 197, referring to § 162.72-2(2)). The use of daphnids or stoneflies or mayflies or any other single aquatic invertebrate may provide no useful information for impact assessment purposes. Daphnia, unless totally destroyed from an environmental niche would be expected to recover so rapidly as to provide no discernable effect for other feeding organisms.

[EPA response: *The Agency disagrees with the comment that no useful information for impact assessment may be provided by testing a single species of aquatic invertebrate for acute toxicity. For example, the concentration of pesticide that is toxic to or may eradicate sensitive invertebrates must be known and considered along with other date (e.g., use and fate data for estimating habitat exposure) to decide if more testing is needed, such as additional species or life cycle studies.]*

Embryolarvae and Life-cycle Studies of Fish and Aquatic Invertebrates (p. 205, referring to § 162.72-4(a)(2)(iii)). It would seem that toxicity or other effects to mammals and birds are rather speculative when

used to warrant life cycle tests on fish. There should be a more accurate and defensible basis than "supposed similar effects." The statement in § 162.72-4(a)(1)(c)(iv) might provide a more accurate basis.

Aquatic Organism Toxicity and Residue Studies (p. 209-212, referring to § 162.72-5). The same comments are applicable to these requirements as for fish in the above statement (page 205).

[EPA response: *The Agency does not agree that significant effects observed in mammalian or avian reproduction or pesticide accumulation in these animals are speculative when used to warrant life cycle or accumulation tests in fish. Other vertebrates have physiological and biochemical systems comparable to those in fish, and when pesticides reduce reproduction by attacking these systems or accumulate in mammals and birds, the Agency must consider this in deciding need for fish life cycle and accumulation studies. See also the request for suggestions for more appropriate, exacting criteria in the preamble, paragraph IV(E).*]

The opportunity to comment has been appreciated.

Sincerely,

PHILIP C. KEARNEY,
Acting Pesticide Coordinator, Office of Environmental Quality Activities.

ADDENDUM II

FIFRA section 25(d) requires the Administrator to provide the FIFRA Scientific Advisory Panel a copy of any proposed regulation at least 60 days prior to signing it for publication in the FEDERAL REGISTER. This provision is to permit the Panel opportunity to comment on the proposal, and it did so for these guidelines and preamble on subparts B, D, and E. The comments of the Panel and the Administrator's response thereto are published below. The page numbers mentioned in some of the comments refer to pagination in the draft (dated Jan. 30, 1978) the Panel was reviewing. For purposes of brevity in this appendix, each specific comment provided by the Panel is followed immediately by insertion of the corresponding EPA response printed in italics and set off in brackets. Readers are reminded that comments referring to guidelines sections beginning "162" should be interpreted to be those that are now "163" in the proposed guidelines.

ENVIRONMENTAL PROTECTION AGENCY,
February 22, 1978.

Subject: Review of FIFRA proposed guidelines for registering pesticides in the United States.

From: Dr. H. Wade Fowler, Jr., Executive Secretary, FIFRA Scientific Advisory Panel.

To: Deputy Assistant Administrator for Pesticide Programs (WH-566).

The FIFRA Scientific Advisory Panel has completed review of proposed subparts B, D, and E of the guidelines for registering pesticides in the United States. Attached is a report of findings by the Panel.

FIFRA section 25 (a)(2)(A) and (d) provide, among other things, that EPA shall not publish a proposed regulation any sooner than 60 days after the Agency has made the proposal available to the Panel for review. These rules also provide that this time requirement may be waived or modified by agreement between the Panel and the Administrator. Accordingly, the Panel agrees to waive the 60-day requirement and agrees that EPA should publish these proposed guidelines as quickly as is consistent with meeting the other obligations imposed by section 25(d) to respond to the Panel's comments.

H. WADE FOWLER, Jr., Ph. D.

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT (FIFRA)

SCIENTIFIC ADVISORY PANEL

Review of Proposed Guidelines for Registering Pesticides in the United States

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel completed review of proposed guidelines for registering pesticides in the United States in open meetings held in Arlington, Va., during the period February 2 through February 3, 1978. The following specific subparts of the guidelines were reviewed during this meeting: Subpart B, Introduction; Subpart D, Chemistry Requirements; and Subpart E, Hazard Evaluation for Wildlife and Aquatic Organisms.

The manner in which EPA Staff personnel developed the proposed guidelines is worthy of special praise. In response to a request by EPA for scientific support while reviewing draft documents, the panel conducted a series of open meetings to mark up a progressively improving proposed guideline document. Following each session, the Agency would evaluate comments from the panel and all other sources and appropriately amend the document on issues no longer in contention. In order for the Agency to obtain additional public comment, some issues remained in contention for several sessions. The panel wishes to commend the Agency for being exceptionally cooperative and responsive to suggestions for improvement.

Maximum public participation has been encouraged at all meetings of the FIFRA Scientific Advisory Panel. In regard to the formal meeting conducted during the period February 2-3, 1978, a FEDERAL REGISTER notice was published on January 18, 1978. In addition, telephonic notice and special mailings were sent to the general public who had previously expressed an interest in the proposed guidelines. Written statements relative to the proposed guidelines were received throughout the informal and formal review sessions from the following sources: Dow Chemical Co. (5 submissions), Wildlife International Ltd., Shell Oil Co. (2 submissions), E. I. DuPont De Nemours & Co., ICI United States, CIBA-Geigy Corp. (2 submissions), Monsanto Co., Onyx Chemical Co. (4 submissions), Union Carbide Corp., U.S. Department of Agriculture, Insect Control and Research, Inc., Velsicol, P.P.G. Industries, National Agricultural Chemicals Association, and Elanco Regulatory Services (2 submissions). In addition to comments by EPA staff, informal comments were recieved during the meeting by representatives of the Environmental Defense Fund, and members of the pesticide industry.

In coinsideration of all matters brought out during the panel meeting, matters articulated in written statements, and a careful review of the proposed rulemaking document, the panel submits the following report:

The FIFRA Scientific Advisory Panel is of the opinion that the three subparts of the pesticide registration guidelines (subparts B, D, and E) submitted to the panel as proposed rulemaking during the period February 2-3, 1978, deals effectively with this procedure with a limited number of specific exceptions. The Agency developed an excellent compilation of specific concerns of the panel. This was articulated in documents transmitted to the panel on January 31, 1978, and February 2, 1978. The documents reflect major and minor changes taken both on the initiative of EPA and as a result of recommendations by the panel during earlier sessions. Overall, the January 31, 1978 document addresses 6 major changes and an attachment with 20 specific concerns with EPA comments on proposed action. The February 2, 1978 document reflects a total of nine minor revisions made by EPA staff as a result of panel recommendations during the meeting on February 2, 1978. All the documents referred to in this report are part of the public record and will be included in the minutes of the formal meeting. Only matters still in contention will be addressed in this report.

The FIFRA Scientific Advisory Panel unanimously submits the following specific comments on the three proposed subparts of the pesticide registration guidelines.

1. *Section 162.40-1(k) Technical revision of the guidelines.* The panel recommends that the guidelines be examined in a public forum periodically (e.g., every 2 years) by a professional group outside the Agency (e.g., NAS [National Academy of Sciences], ASTM [American Society for Testing and Materials], to see that the guidelines keep pace with available technology and current needs. The EPA response that the Agency is committed to periodic review as noted in the preamble on page 6 is not sufficient.

Panel Recommendation: The language of section 162.40-1(k) be changed to reflect the intent of the panel that the documents should be subjected to periodic technical review to ensure currency with scientific technology and needs (normally reviews should be held at 2-year intervals).

[EPA response: *The language of § 162.40-1(k) states: "The guidelines and appendices will be expanded and revised periodically to reflect new scientific knowledge, new trends in pesticide development, and new Agency policies." The Agency believes that this statement adequately reflects the Panel recommendation.*]

II. *Subpart D, Chemistry requirements.*

A. *Section 162.61-4 Description of manufacturing process.* From the standpoint of hazard evaluation, description of the manufacturing process is redundant in view of the requirements of 162.61-6, Declaration and certification of ingredient limits. *Panel recommendation:* That the Agency reconsider its requirement for extensive data on the manufacturing process of pesticides and modify or delete requirements in section 162.61-4 to include only data which is needed to make a hazard evaluation.

[EPA response: *A sentence has been added in response to the Panel's recommendation: The information need only include those factors which affect the composition of the product and its certified limits on ingredients."*]

B. *Section 162.61-6 Declaration and certification of ingredient limits.* Should be modified for consideration of exclusions. *Panel recommendation:* That the Agency provide more definitive language for exclusions or devise a system whereby exclusions can be considered, such that registration applicants will not be supplying composition information and certified limits on chemicals of no real importance in Agency hazard evaluations.

[EPA response: *The Agency is in the process of determining which information on product composition and certified limits on ingredients would have no real importance in the Agency's hazard evaluation. Until final determinations are made, the Agency believes that the requirement should remain as is. Public comment may supply some appropriate criteria.*]

C. *Section 162.62-4 Disclosure of professional qualifications* of testing personnel should be on a reciprocal basis with Agency reviewers. *Panel recommendation:* That the Agency indicate in some public statement that the public has the right to request the professional qualifications of agency personnel (such as registration scientific reviewers). This is in response to the provision of the guidelines which states that the Agency may require resumes of the qualifications of testing personnel (162.62-4(a); 162.70-1(b)(11)). This should be done on a reciprocal basis with registrants.

[EPA response: *The Agency can release resumes of Agency personnel, upon request, to the extent permitted by the Freedom of Information Act and the Privacy Act.*]

D. *Section 162.62-7(c) Data requirements: Physico-chemical degradation, photodegradation. Panel recommendation:* That the Agency consider deleting the requirement for pesticide photodegradation data in soils, since too many other factors are also concurrently responsible for degradation in soils. Thus, the data in most instances will be difficult to interpret with precision.

[EPA response: *The Agency recognizes the limitations that must be considered in evaluating data on photodegradation, but has decided to retain this proposed requirement to elicit further—hopefully constructive—public comment.*]

E. *Section 162.61.7 Product analytical methods and data.* The panel is specifically concerned about the requirement for quantitative limits of 0.01 percent for impurities discussed in sections 162.61-7(a) (2), (3), and (5). *Panel recommendation:* That the word, "identifiable" be inserted prior to the word *impurity* in sections 162.61-7(a) (2), (3), and (5). There was ambivalence by members of the panel concerning the quantitative limits of 0.01 percent for identifiable impurities. The position of the panel on this issue is that the Agency should consider seeking an intermediate position between 0.1 percent and 0.01 percent that is consistent with scientific technology for detecting impurities. The panel respectfully requests that all concerned parties submit data to the panel on this issue during the comment period, with the view of commenting on a specific quantitative limit for impurities in the final document.

[EPA response: *The term "identifiable" has been inserted in the 3 locations specified by the Panel. Public comments on this and any other proposed requirement should be submitted to the address specified in the beginning of the preamble to the guidelines. All comments received will be made available to the Panel.*]

III. *Subpart E. Hazard evaluation: Wildlife and aquatic organisms.*

A. 162.71-1 Avian single-dose oral LD50 and 162.71-2 Avian dietary LC50.

1. The usefulness of gross necropsies data is in contention. *Panel recommendation:* The panel is of the opinion that data obtained from gross necropsies (pages 150, 156, and elsewhere) are not useful for the purposes of the avian dietary LC50 or the avian oral LD50, and should be deleted from the list of requirements.

[EPA response: *In each instance where gross necropsies are mentioned in this subpart, they are part of a suggested protocol and not officially part of the guidelines requirements. On "page 150" of § 162.71-1, the statement says "Gross necropsies could give valuable additional data but are not necessarily required"; this is certainly not a requirement. On "page 156" of § 162.71-2, the statement says "A gross pathological examination shall be conducted on sufficient birds to characterize the gross lesions in dead birds". In both instances, the Agency feels that a certain amount of very good information can be derived from necropsies of such birds (provided the tissues have not autolyzed significantly). Therefore, these guidelines statements have not been deleted.*]

2. *Age of Birds.* The difficult problem of selection of ages of birds to be tested (pages 153 and 165) was not resolved. Comments during the meeting were useful and a further attempt at a compromise should be made by the Agency during the public comment period. *Panel recommendation:* The Agency solicit additional public comment on this issue and appraise the panel of results achieved prior to review of final rulemaking.

[EPA response: *The Panel is referring to sections 162.71-2(b)(3) and 162.71-4(b)(4). The Agency agrees with the panel recommendation.*]

B. *Section 162.72-5 Aquatic organism toxicity and residue studies.* The location of bioaccumulation tests poses a difficult problem. The panel found persuasive arguments for location in both the environmental chemistry (162.62-11(d)) and in this section. *Panel recommendation:* The Agency reconsider the proper location in the guidelines for bioaccumulation tests and appraise the panel prior to review of final rulemaking.

[EPA response: *The Agency intends to reexamine the nature and location of these test requirements in the guidelines after public comments have been received.*]

For the Chairman.

H. WADE FOWLER, Jr.,
Executive Secretary,
FIFRA Scientific Advisory Panel.

[FR Doc. 78-18705 Filed 7-7-78; 8:45 am]